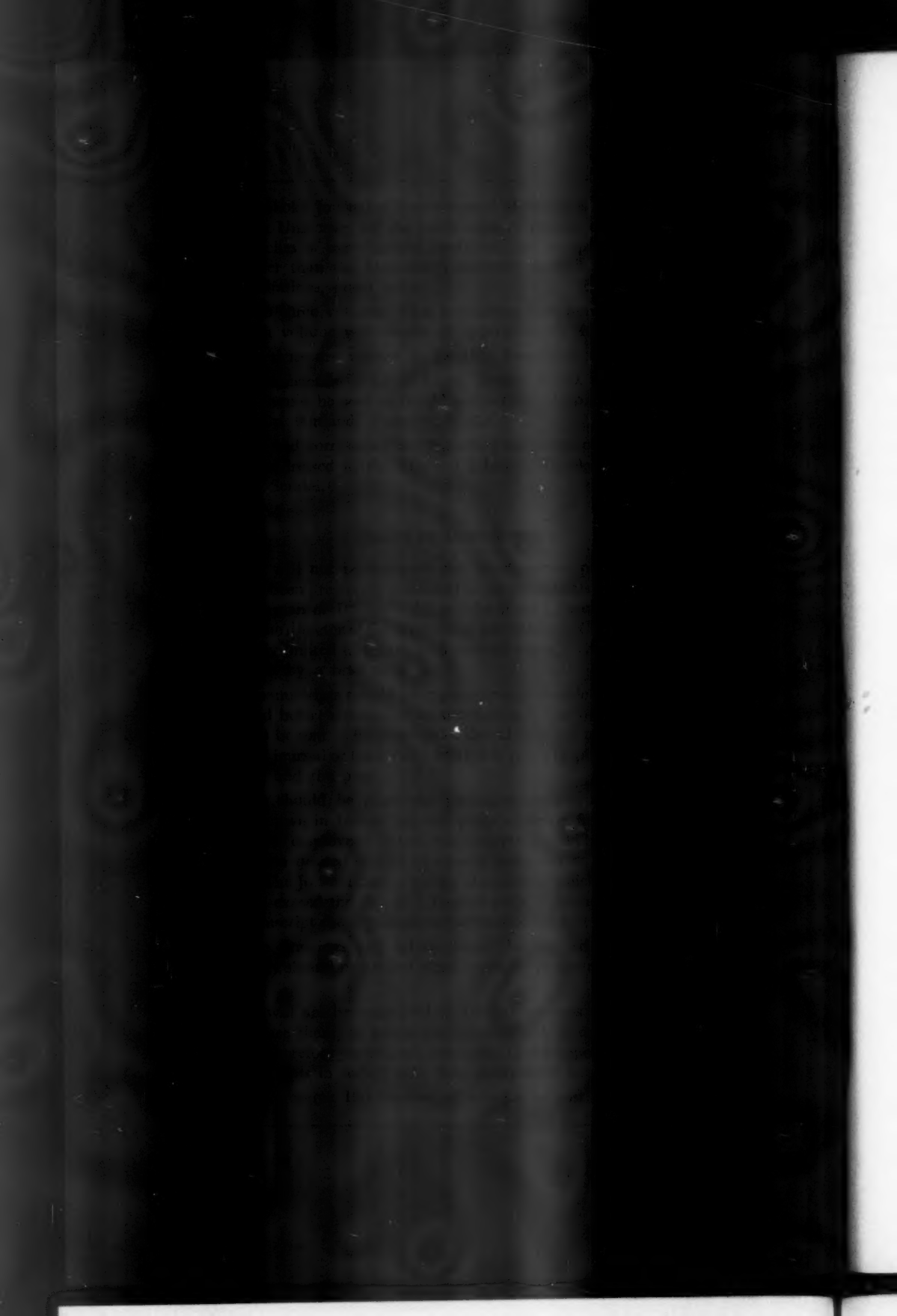

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STUDIES ON MELANIN

By

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PREFACE

I came to the Department of Dermatology of the Tohoku University Medical Faculty in 1937. Selecting the subject of melanin as one of the main themes for extended study, my staff and myself devoted much of our efforts to the clinical, histological and histochemical examination of the various pigmentary affections of the skin as well as series of experiments with animals on the various melanin productions.

As we progressed with our studies and experiments, we gradually reached certain basic deductions regarding the mechanism of melanin production. Thereafter, based upon these deductions, we were able to plan and carry out our experimental works and diagnosis with certain objectives in mind. In the course of our studies we also discovered that among the Yellow races there are types of pigmentary affections different from those found among the White and Colored races, which fact, we believe, well facilitates the melanin studies.

Our researches have been published from time to time in medical journals in Japan. However, during and following the last world war, publication became extremely difficult, making it practically impossible for us to publish enough of the materials and documentary evidences, especially the photographs and other illustrations. Consequently, much to my distress, we were not able to express our findings and theory in print and communicate them fully to the academic circles.

Finally, however, with the willing cooperation of my colleagues and particularly with the Grant in Aid for Publishing Research Result from the Ministry of Education, we have been able to compile the complete results of the many years' studies in the present issue of the Supplement to the Tohoku Journal of Experimental Medicine. Several articles of this issue have already been reported as rough notes on this Journal but they are explained here in detail for the purpose of fully understanding of readers. We hope that our studies during the long period of academic isolation have kept pace with the similar studies in the other parts of the world, and also sincerely hope that our readers will kindly communicate to us their frank criticisms and suggestions on the present treatise.

My cordial gratitude is expressed to the coworkers Drs. Hamada, Iijima, Nakajo, Watanabe, Yoshida K. and Yoshida Y. for their great efforts in respective branch fields of the present investigation.

MINOR ITÔ

Sendai
October, 1951

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I. Introduction*

The mechanism of melanin production in human being as well as in all zoological circles has been a long disputed problem. The study on this point began to attract much attention and interest towards the end of last century. Especially in 1917 Bloch[1] proposed his famous Dopa theory which clearly offered a solution both to enzymological and histogenetical problem. This theory has since been the center and impellent force of researches in this field. According to Raper and Evans,[2] the chemical process of melanin production takes place in the following manner. First tyrosine is converted to dopa by the action of enzyme tyrosinase, and dopa in turn is oxidized by dopa oxydase to dopa quinone which undergoes further oxidation and polymerization and finally turns to melanin. This theory materialized the dualism of Brourquelot and Bertrand. However, the true nature of melanin and the actual chemical process of melanin production cannot be explained solely by these theories, and especially the existence of the enzyme dopa oxydase is still disputable and recently single enzyme theory is again proposed by Keilin and Mann,[3] Lerner and Fitzpatrick,[4] Calkins and Summerson. Moreover recent works point to the investigation of biochemical condition in pigment production such as activator substance (copper[5] and iron[6]), glutathione, ascorbic acid, hydrogen ion concentration[7] and redoxpotential[8] and so on.

The participation of endocrine system and vegetative nervous system in melanin production esp. in the change of body color has been suggested by many observations on animals. These investigation results, however, cannot be readily applied to human being.

The improvement in the technics of histological staining resulted in some progress in this field. On the origin of nevus cell the drop theory of Unna is no longer accepted. The neuroectodermal theory of Masson[9] appeared instead. On the problem of malignant melanoma, numerous new findings are reported, which are summarized in the special issue of New York Academy of Science under the title, "The Biology of Melanomas." But the nosological nature of this tumor is still under discussion. So in many cases it is impossible to decide whether one is dealing with a cancer or a sarcoma, and such cases are called vaguely melanomalignoma according to Miescher.[10] Therefore, in the present state of researches it is quite natural that we cannot always account for the pathogenesis of pigmentary disturbances following various dermatological and endocrine disorders.

Our first step was to find the genesis of nevus cell with Seto's improved method of Bielschowsky's silver impregnation of neurofibrils. In this investigation the Schwannian cell theory of Masson was ascertained. Then this method was applied in combination with Bielschowsky-Maresch's argentaffin fibril staining in the histological study of various pigmentary disorders. In Mongolian spot, blue nevus and in nevus fuscocaeruleus ophthalmomaxillaris of Ota, the mesodermal origin of mela-

* By Minor Itô.

noblasts was confirmed and consequently these three lesions were attributed to same pathogenetical system closely related to nervous system. This forced us to concentrate our attentions to the study of relationship between pigment production and nervous system. In clinical observation histamine scratch test and diaphoretic test were performed in order to verify homolateral nervous disturbance in loco. The results were estimated to be indirect evidence of nervous regulation of pigment metabolism. Further, dysharmony of autonomic nervous system was examined in individual case.

While on the other hand various histochemical technics were applied in sections of pigmentary disorders. The results of dopa reaction, oxydase and peroxydase reaction, Unna's oxidoreduction staining[11] led us to a certain conclusion in the biological state of the site of pigment abnormalities. Especially the origin of malignant melanoma was studied in some detail by these methods. The manifestations of vital oxidoreduction were studied in animal experiments, the results of which together with the above mentioned clinical findings, helped us in the research of melanin production.

The rôle of endocrine secretions, ascorbic acid and riboflavin in acquired pigmentary disorders especially in Riehl's melanosis and in vitiligo was discussed. Their participations in melanogenesis were interpreted in the sense of hormozym or vitazym (Euler[12]).

In the genetical study of skin diseases, which is another main theme of my clinic, the survey of genetical pigmentary affections was also involved. According to the physiologic-genetical conception of Goldschmidt, the heredity of Mongolian spot, freckles, symmetric hereditary dyschromatosis and xeroderma pigmentosum[13] are found to be polymeric dominant with two factors, melanogen and oxydase.

The aim of our present study was partly to find a definitive therapeutic procedure of pigmentary diseases especially of vitiligo, in which respect, we regret, we still have not yet been successful.

Throughout our work we were able to see evidences of the participation of nervous system in melanin production. Therefore much importance was attached to this point in all the articles resulting inevitably in simplicity of description on other subject.

Works from chapter II to XV consist chiefly of clinical investigation results, while those from XVI to XIX contain experimental results. The illustrations in each chapter are collected on the end of present issue, and the microphotographic illustrations are minified for the sake of convenience and their magnifications are not mentioned.

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II. Genesis of the Nevus Cell*

Research on the genesis of the nevus cell in pigmented nevi is being carried on by many investigators such as the Fibroblast theory (Simon, Rokitanski and Virchow), the Bloodvessel-wallcell theory (Demieville), the Lymphatic-vessel-endothelialcell theory (Recklinghausen) and the Chromatophore theory (Ribbert). These accentuate the mesenchymal origin. But when the Abtropfung theory was proposed by Unna, it was accepted by many investigators, especially on account of the dopa reaction of Bloch[1] that leads to the theory of the skin nerve origin of Masson[2] which is being much discussed.

I have also made a study of the above subject. First of all, I concentrated on the examination of the relationship between the skin nerve and nevus cells by the advanced method of Bielschowsky's silver impregnation of neurofibril (Seto,[3] Tohoku University) and then discussed the problem of fiber formation of nevus cell by the Gitterfaser staining of Bielschowsky-Maresch.

Materials were taken from 13 patients and were fixed at least 10 months in neutral formalin (Seto's method). Frozen sections about 30 μ in thickness were made and stained. At the same time, the histology of neurofibroma, neurinoma, malignant melanoma, blue nevus and Mongolian spot was studied by the same method.

Microscopic studies of the Bielschowsky-Seto's neurofibril staining preparations: At nevus lesions the neurofibril bundles are prominent at the deeper layer of the cutis under low power microscope, reminding one of the polyneurie of the French school. At places where nerve bundles penetrate or run close to nevus cell nest, their Schwannian cells are much increased in number. The nuclei of these Schwannian cells are so hypertrophied in a circuloid form that the nerve bundles seem to swell as a whole mixed with nevus cells, and consequently it is difficult to differentiate the nevus cell nucleus from that of the Schwannian cell either morphologically or dispositionally. The fine ramificated neurofibrils coil round the nerve bundle like a gabion, and their Schwannian nuclei give the impression of transition to nevus cells (Plate I, Figures 1 & 3).

More or less in all specimens neurofibrils running into the nevus cell nest of reticulated midlayer are found, and especially in several preparations the cross and oblique sections of nerve bundles are visible. There is the particular figure of several looped and ramificated nerve bundles between nevus cells. The nuclei of Schwannian cells are typical long-oval and bend together with neurofibrils but some nuclei are so swollen that we can not distinguish them from nevus cells (Plate I, Figures 2 & 4).

Several magnitudes of nevus cell nest and single nevus cell are situated sporadically and they come into contact with the course of one or two neurofibrils and fine vegetative nerve (Plate II, Figures 5 & 6).

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* By Minor Itô. Published in this Journal, 1950, **52**, 95.

In several specimens of young children suggestive of early stage, nevus cells exist diffusedly in the spongy tissue without the nest formation. The shapes of the cells are round, oval, comma-shaped or rarely stellate and some of them are connected with each other with their dendrites. Fine bundle of neurofibrils and nevus cell groups situated in the same course with neurofibrils is found among them. The formation syncytiale which was pointed out by Miescher was noticed everywhere in nevus cells. Sometimes the Schwannian cell and the nevus cell are twisted with each other and this fact shows the morphological transition between these two cells. Moreover in the bluish-brown colored portion of the large pigmented nevus over the breast of a 20 years old female, the groups and cords of nevus cell are found in upper cutis and the infiltrative growth of nevus cell was noticed in midlayer of cutis. Furthermore in the specimen obtained from the gigantic nevus on the thigh of a 22 years old female nevus cell showed the nest formation in subpapillary layer and the neurofibromatous structure in reticulary midlayer (Plate II, Figure 7). That is to say, the figure of contemporal existence of socalled Type A, B and C of nevus cell which were advocated by Miescher is found in the same specimen. In my observation of several specimens, I recognized the figure of something resembling nerve-terminals (Plate II, Figure 8), on which Masson based his nerve cell theory, *i.e.*, morphology of fibres naeviques, faisceau neuroide, lames foliacées, corpuscules naeviques and cellules claires in basal membrane.

The blood vessels are found near or in nevus cell nest but I have not been able to find any figure which shows the relationship between these vessel-wallcells and nevus cells or their transition in any part. The Abtropfung figure is refuted by the following observations of Gitterfaser staining slides.

Microscopic studies of the Bielschowsky-Maresch's Gitterfaser staining preparations: Results of observations are similar in all preparations, *i. e.*, the nevus cell nests are completely packed with argentaffin fibrils which penetrate into the nests and show the fine reticulated appearance, and each nevus cell looks as if it is inlayed in each reticulum (Plate V, Figure 18). Especially at some places where nevus cells are scarcely located, closely united figures of argentaffin fibrils and nevus cell protoplasma, and also the nevus cells, abundant in melanin, sticking to argentaffin fibrils botryoidally, are indicated distinctly. However, in the central part of big nevus cell nests in the superficial layer, the argentaffin fibril is not clearly stained out.

On the other hand, the attitude of argentaffin fibrils toward the epidermal layer is utterly different from the above, for they confront with each other decidedly. The minutely rugged argentaffin fibrils are located separately from fine clear non-stainable zone (socalled hyalinous membrane of Ranvier). Particularly at regions where the nevus cell nest is projected against the epidermal layer, the fine argentaffin fibrils draw a line between these two. Argentaffin fibrils show a clear demarcation against sebaceous glands and sweat glands by a smooth line, or pretty nevus cell nests which are packed in chrysanthemum-like reticulated picture observed around the oblique sections of hair follicle. Therefore, as the unclear point with regard to the relationship between the nevus cell and the epidermis in former neurofibril staining preparation becomes clear by Gitterfaser staining, I do not believe that Abtropfung in the sense of Unna can be upheld. Moreover, I have confirmed these observations by Mallory's staining.

Comment

Relationship between nevus cell and skin nerve: In 1899, Soldan advocated that nevus cell belongs to a tumor of connective tissue cells originated from neurofibril sheath, but people took no notice of his opinion as just then the Abtropfung theory of Unna was becoming influential. However, in 1926 Masson[3] established the neural origin theory of nevus cells that was founded on the observations by his own method called Trichrome staining. According to him, the nevus cell comes from cellules claires that corresponds to the Merkel-Ranvier's tactile cell in the epidermis. He pointed out the fibres naeviques, faisceau neuroide, lames foliacées, corpuscules naeviques in the cutis and considered the nevus cell as a special form of skin nerve which has lost its sheath and axis cylinder but has proliferated Schwannian cell. This excellent new theory called attentions of the investigators. Chandler Foot[4] first supported it, Miescher and Albertini[5] made a supplemental experiment of Masson's method and they both helped to uphold the new theory. Then Feyrter,[6] suspecting its origin to be the endothelcell-like Häutchenzelle which is located in the external sheath of nerve, recognized this fact by a special myelinsheath staining. John[7] continued the study by the Bielschowsky-Gros's method and noticed the swelling and karyocytosis of Schwannian sheath as the abnormal course and ramification of neurofibril in nevus cell nest. He considered them as a manifestation of periferal nervous reaction which concerns the whole nerve tissue and noticed the Stalagmocyte in the epidermis, a type of proliferation and agreed to the bipolar theory. Ota[8] obtained analogous results to that of Masson, and therefore, advocated bipolarity.

I made clearer preparation by Seto's excellent modification. On account of the nature of the nerve tissue, I am unable to regard the abnormal figure of neurofibrils in nevus cell nests to be a simple incidental or passive transformation, but I am inclined to believe that it has some active or causative relationship to the nevus cell formation. Especially in Plate I, I can see the process of transition of Schwannian cell of neurofibril to nevus cell by the twisting of the nerve bundles in the deeper layer like a gabion. I believe the above observation is the best evidence which supports the theory of Schwannian cell origin of Masson.

Relationship between nevus cell and argentaffin fibrils: Demieville, Dalla Favera, Bauer, Löwenbach, Abesser, Ribbert, Oppenheim and others proved that there are fibrous substances around the nevus cells which penetrate into the nest reticulately. With regard to the origin of these substances, Dalla Favera, Takeuchi and others assumed that the nevus cell infiltrates into collagenic fibers, while Unna, Abesser, Fick and others considered the substances as a new born fiber from existing connective tissue. However, a school which maintains the mesenchymal origin of nevus cells (Recklinghausen, Bauer, Scheuber, Ribbert, Krompecher, Borst and Hausemann, Fischer and others) regards the fibrous substances as a product of nevus cells. But Kromeyer, together with Judalewitsch, suggested that the epidermal cells first drip down and then change into the connective tissue. He advocated this phenomenon as Desmoplasie. Masson advanced the theory that the fiber originated from the substratum of Schwannian sheath. Then Lucioni, Cohn, Chandler Foot, Feyrter and others recognized that the nevus cell is packed with reticulated fiber by several

silver impregnations and advanced their own theories.

If we are to consider the argentaffin fibrils as the Gitterfaser in a narrow sense, my observation leads us to call our attention to the reticuloendothelial tissue and it becomes favoraole to the mesenchymal theory and conflicts with my own observation in the foregoing paragraph. But we can not consider the argentaffin fibrils to be always mesenchymal, especially, since Sekine[9] explicated recently in his embryomorphological study that there exists neurogenic variety of argentaffin fibrils as well as mesenchymal and epithelial ones. So he recognized that the Neurilemmafaser is no other than the Schwannian sheath and that this argentaffin fibril is separated from the collagenic fiber of internal sheath and therefore, held that it should undoubtedly belong to the neuro-ectodermal origin. On the other hand I identified the argentaffin fibril of the Schwannian cell on the Bielschowsky-Maresch's staining preparation of neurofibroma and neurinoma which I studied for the sake of consultation. It can be affirmed that the argentaffin fibril in my slides of nevus cell is the neurogenic fibril which originates from the Schwannian cell, and consequently, the theory of Soldan and Feyrter that maintains the origin to be the internal and external sheath of neurofibril can be refuted at the same time. But the parade-like arrangement such as seen in a neurinoma is scarcely found in nevus cells. This is easy to understand when we think of the difference between neurinoma which comes out in non-peripheral nerve bundle, and nevus cell which is a lesion at the periphery of skin nerves. The attitude of argentaffin fibrils on the epidermal layer is very clear as reported previously. Even at places where nevus cell nests come close to the epidermal layer, I have always maintained the presence of argentaffin fibrils. This fact corresponds to the presence of the socalled Randbindegewebe between nevus cell nest and epidermis which was noticed by Bauer[10] and traced by Demieville, Ribbert, Wieting and Hamdi, Soldan, Berblinger and others. I note such fact as a counter-evidence of Unna's Abtropfung theory.

Relationship between nevus cell and melanin: In accordance with the Abtropfung theory of Unna, the Dopa-oxydase theory of Bloch seems to be convincing of the ectodermal origin of nevus cells. But there is much doubt with regard to the theory, as Kissmeyer, Kaiserling and others have already pointed out. Moreover, the instance of dopa-positiveness in blue nevus and Mongolian spot observed by Sato and Bahrawy of Bloch's school is not necessarily favorable to epidermal theory and I believe I can point out the contradiction of Unna's theory in my study[11] of blue nevus and Mongolian spot, which I believe to be of mesenchymal nature.

Inclusion of melanin is not necessary in nevus cells, although abundance of melanin is generally found in the superficial nevus cells and it can also be found in the deeper cell nest or in scattered nevus cells. This fact is unexplainable as a photodynamic reaction and we are to find the new signification of dopareaction in this point. And I observed by Bielschowsky-Seto's staining preparation the vegetative neurofibril and its terminal reticulum, paticularly at parts where melanin is found in nevus cell nest or in scattered nevus cells. I also noticed such contact of vegetative neurofibril in the comparative study of Mongolian spot, blue nevus, malignant melanoma.[12] Therefore it is evident that there exists some relationship between vegetative nerve fibrils and melanin production. Namely, if a cell, be it a nevus cell of neuroectodermal origin or mesenchymal chromatophore (as it is the case with a

Mongolian spot), has some contact with the termination of vegetative nerve fibrils, then the cell is possessed of melanogenic facility. Consequently, it may be assumed that melanin production is the result of the chemical transmission of vegetative nerve in the socalled Erfolgzelle, or shortly, a neurohormonal reaction (Vilter). Therefore it is proper to assume the participation of oxydase. The dopareaction, I conclude, is no other than a sign which reveals the secret of this process.

Summary

By means of the Bielschowsky-Seto's method of neurofibril staining, the author have concluded that the nevus cell is not the production of the "Abtropfung" of epidermis (Unna), but originates from the neuroectodermal cell which is homogenetic with the Schwannian cell (Masson).

I have recognized the argentaffinity of the Schwannian cell by Bielschowsky-Maresch's staining, and have supported the neurogenic nature of nevus cell. But I am obliged to reject the socalled Abtropfung theory on account of the indifferent attitude of argentaffin fibril against the epidermis.

The author believes the melanin production depends entirely upon the chemical reaction in the cell which is in contact with the termination of peripheral vegetative nerve, and that it does not matter whether the cell is ectodermal or mesodermal.

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III. Histochemical Investigation on Nevus pigmentosus*

It has been recognized that melanin is the last product in the process of oxidation which is caused by catalysis of ferment. By the report of Itô & Nakajo[1] and Itô & Watanabe,[2] it is evident that melanin production is experimentally possible by transportion of a substance by which we can provoke the biocatalysation. In that case, what kind of and how many ferments in the living body take part in the melanin production? On this question, yet many different opinions are advanced. We have examined indophenolase and peroxydase on this relationship in melanin production.

Histochemical Examination on Oxydase and Peroxydase Reaction

Bloch and Ryhiner[3] reported that protoplasm of epidermis or hair sheath cell gives negative indophenol-bluereaction but positive dopareaction. But Meirowsky[4] said that the melanoblast becomes colored under the existence of chromogens (for instance, dioxynaphthalene, *p*-amino-salicylate, *p*-phenylendiamine, etc.). Gräff[5] authenticated that basal cell shows also positive nadi-reaction; Katsunuma,[6] Okano[7] described that in epidermis, external hair sheath and especially in Malpighian layer or basal cell, this reaction is most evidently positive; Shiga[8] reported that dopareaction on skin coincides with the oxydase reaction.

Examining the peroxydase reaction histochemicaly, confirmed Unna[9] that the all nuclei and the granules in the protoplasma of mast cell are peroxydase-positive and from this fact, he concluded them as special socalled "Primäre Sauerstofforte" (primary oxygen area). But Okano does not always agree entirely with his opinion. On this chapter, we state the histochemical investigation of both reactions mainly in the melanoblast.

Materials: 14 cases with nevus pigmentosus or Mongolian spot and skin of guinea-pig were put to use.

Methods: "Gewebes-Nadi-Reaktion" is applied as the labile oxydase reaction; in the stabile peroxydase reaction, the objects are fixed in alcohol and celloidin sections are made. We followed Sato-Sekiya's[10] copper method because of unstable results of Unna's peroxydase method and accomplished our purpose for the most part. As for labile peroxydase reaction upon frozen section, Okano[7] C method is applied. Reagent used consists of equal part of saturated benzidine aqueous solution and hydrogen peroxyde solution. In addition, the oxygen area—"Sauerstofforte"—and the reduction area—"Reduktionsorte"—are examined by means of permanganate methylgreen method of Unna and at the same time all the cases were stained with hematoxylin-eosin as comparisons.

Results of experiments: 1) Labile oxydase reaction is positive in protoplasm of Malpighian layer of epidermis, external hair sheath, endothelium of bloodvessel and capillary, cells of coil-glands and its ducts, and nevus cells (Plate III, Figure

* By Minor Itô and Masahiko Watanabe.

9). Above all, lower layer of rete Malpighii, external hair sheath, nevus cells and coil-glands give the most strongly positive reaction. Capillary endothelium in the upper layer of corium, isolated melanoblast, etc. are also strongly positive but chromatophores give always negative reaction, although some of them show slightly positive reaction. In the black fleck of guinea-pig, lower layer of epidermis gives strongly positive reaction.

2) Melanin granules of all kinds of abovementioned pigment cells show negative oxydase reaction; moreover oxydase granules in the pigment cell are all the same to the proper melanin granules in size and in shape.

3) Peroxydase reaction is positive in all nuclei (Plate III, Figure 10); especially basal cell of epidermis and of external hair sheath, nevus cell, coil-gland and its duct, capillary and bloodvessel endothelium give strongly positive reaction. Melanoblast and chromatophore in Mongolian spot are sometimes considerably positive, but the most part of them is negative.

4) When the peroxydase reaction is strongly positive, in addition to the homogeneous tint of blue of nuclei, the diffused or granular staining in protoplasma is often observed. Such a observation was often seen in basal layer of nevus pigmentosus, in nevus cells in the upper layer of corium (Plate III, Figure 11), in chromatophores and Mongolian spot cell, and also in lower layer of epidermis and in dermal pigment cells in guinea-pig skin (Plate III, Figure 12).

5) Pigment bearing cells have a tendency that the much more melanin granules they contain, the less becomes the percentage of granules which are oxydase-positive or peroxydase-positive.

6) Examining histochemically oxygen area and reduction area by Unna's method, we observed in the basal layer of epidermis and in the external layer of nevus cell nest of corium, that the area where melanin granules to be seen abundant was stained in blue; that is socalled "Sauerstofforte." To the contrary, in the inside of nevus cell nest, the area where the melanin granules were absent, was stained in faint-brown with potassium permanganate; that is "Reduktionsorte" (Plate III, Figure 13). It indicates precisely the biochemical changes of these areas, and we suppose that in accordance with these changes, melanin production is influenced. As 3 cases of vitiligo which is examined as the control, in papillary and subpapillary layer the "Reduktionsorte" is more intense in the white fleck as compared with that of surrounding normal skin; this result is a good contrast to the abovementioned results.

Indophenol-oxydase and Peroxydase Reaction on the Skin Extracts of Guinea-pig

The skin extracts of black and white fleck of young guinea-pig were studied comparatively.

Materials of experiment: Preparation of skin extract.—Hair of a guinea-pig was cut off as closely as possible. No sooner the carotid artery was cut and bloodletting was done, that guinea-pig was skined. Then adipose tissue, connective tissue and bloodvessel were removed; then it was cleansed with physiological salt solution; and then the black fleck was separated from the white one, and 5 g. of quartz-sand was added separately to 10 g. of both of them and rubbed them into porridges. Then distilled water was added to each porridges and shaked up to emulsion until

either of them became 20 cc. in all. After stored in refrigerator, both of them were separated by centrifugalization. And we made use of their clear supernatant fluids.

Methods: We followed Staemler & Sander's[11] and Mori's[12] technic; Oxydase and peroxydase of each black and white skin extract are contrasted by Dubosq's colorimeter. Examining oxydase, indophenol-blue which is produced from the following procedure is used, that is, dimethyl-para-phenylendiamine-hydrochloride and *α*-naphtol are mixed with the skin extract of both black and white skin then xylol is added and the colored xylol is contrasted colorimetrically with fuchsin solution. Peroxydase is examined by the benzidine-guajacol method.

Results: As showed in Table I, in both reactions the extracts of black fleck give more darker tint than of white fleck. From these results, we noticed that each extract exactly has much ferment.

TABLE I

Indophenol-oxydase and Peroxydase Reaction with Skin Extracts of Guinea-pig*

Number of animals	Oxydase reaction			Peroxydase reaction		
	White skin	Black skin	Difference	White skin	Black skin	Difference
1	26.0	16.4	9.6	30.4	25.3	5.1
2	29.5	20.9	8.6	25.0	18.7	6.3
3	30.0	26.5	3.5	29.5	22.3	7.2
Mean value			7.3			6.2

* These values show the reading of colorimeter.

Peroxydase Reaction of Local Blood

Sato and Yoshimatsu[13] found such a blood picture in typical cases of acute lethargic encephalitis in which all the myeloid leucocytes of the smear have become peroxydase-negative, while at the same time they remain oxydase-positive. Besides they succeeded in the experiment on rabbit, in which they could observe the same blood picture. And they termed it the striatal blood picture. Sato and Tokue[14] confirmed that the striatal blood picture can be observed in the blood of *Gaudina chilensis* J. Müller in normal condition. In addition, they confirmed the diminution of peroxydase in B-avitaminosis patient.

We surmised that the intensity of the blood peroxydase reaction is related to the biological oxydo-reduction process, and therefore it also has a relation to the pigment production. On this supposition, we examined the picture of local blood in the skin.

Materials: We used smear made out of local blood. Such local blood was obtained from nevus pigmentosus, vitiligo, and from the white and the black flecks of several animals.

Methods: The local skin was previously cleansed with ether. At first, the skin was flayed superficialy as possible, and then the first drop of the blood gushing out dottedly was catched and was smeared homogeneously and thinly as possible on a corner of a slide. For the sake of contrast, blood taken from a ear-lobe by tapping was smeared on the other corner of the very same slide. Method of staining was the

same as Sato-Sekiya's[10] original method. And we calcurated every 100 neutrophile leucocytes of each case.

TABLE II

Peroxydase Reaction of Local Blood of Patients

	Number of patients	Relation to the normal skin		
		Stronger	Equal	Weaker
Nevus pigmentosus	50	31	14	5
Vitiligo	15	2	1	12
Albinism	2		1	1

TABLE III

Local Blood Peroxydase Reaction at Flecks of Several Animals

	Number of animals	Black skin > White skin	Black skin = White skin
Rabbit	6	5	1
Dog	5	2	3
Guinea-pig	5	3	2
Flatfish	5	2	3

Results: The local blood of nevus pigmentosus gives strongly positive peroxydase reaction as compared with that of ear-lobe; to the contrary, the local blood peroxydase reaction of vitiligo has a tendency to be less strongly positive than that of ear-lobe. Moreover in rabbit, dog, guinea-pig or in flatfish, comparing the colored skin with the white skin, we confirmed that the former is more strongly positive than the latter. Even contrasting with each other, however, the difference of intensity of the reaction is so delicate as we could discerne only by means of the shorttimed peroxydase method.

Influences of Various Preparations upon the Blood Peroxydase Reaction

Sato[13] could not observe the striatal blood picture in acute or chronic intoxications, but introducing various colloid substances into the blood stream Suzuki[15] could confirme that the blood peroxydase reaction was suppressed. We observed in human body and in guinea-pig the influence of some medicines which have relation with biological oxydo-reduction upon the blood peroxydase reaction.

From the above, we learned that immediately after intravenous injection of vitamin C and hypertonic glucose solution into the human body, or intracardial injection of vitamin C into guinea-pig or hypodermic injection of adrenalin, peroxydase reaction of blood leucocytes was temporarily suppressed. Besides, 24 hours after the subcutaneous injection of hydroquinone into guinea-pig, local blood peroxydase reaction of black fleck in guinea-pig has been suppressed remarkably. By injection of *p*-benzoquinone, the local peroxydase reaction of the white fleck was somewhat increased.

TABLE IV

Influences of Various Preparations upon Human Blood Peroxydase Reaction

Preparations injected	Number of persons	Before injection	After injection 8′	15′	30′
Vitamin C 4 cc. with 25% glucose 20 or 40 cc. intravenously	14	++	+	+or±	
	3	++	++	++	
0.1% adrenalin 0.7 cc. subcutaneously	6	++			∓or−
	2	++			++
1% pilocarpine 0.7 cc. subcutaneously	2	++			++

++ strong as normal.
+, ±, − weaker than normal.

TABLE V

Influences of Various Preparations upon Blood Peroxydase Reaction in Guinea-pig

Preparations injected	Number of animals	Before injection	After injection				
Vitamin C 10 cc. intracardially	3	++	8′ −or+		15′ ±or++		30′ ++
0.1% adrenalin subcutaneously	2	++	5′ +	10′ ±	15′ ∓	20′ +	30′ ++

COMMENT

From the above results of the histochemical oxydase and peroxydase reaction and the observation of Unna's " Sauerstofforte " and " Reduktionsorte " and of local blood picture, and the intensity of the oxydase and the peroxydase reaction of skin extracts, we confirmed that the both enzymes or local biological conditions are closely connected with the pigment production. Wohlgemuth and Klopstock verified that in the pudendal skin, there is much more polyphenoloxydase than in the other part of skin. Inquiring into tissue culture, Francescon confirmed that though the most part of pigment granules is oxydase positive but dopa reaction is weaker in the premature cell than completely differentiated cells. Luna reported that the pigmentation brings about on *Drosophila* as soon as it is hatched from puta, and that at the same time the peroxydase reaction becomes positive. Mawas[16] verified that the peroxydase reaction is positive in extracts from melanoma.

About the intensity of oxydase on which sunlight and pigment production were concerned, Wohlgemuth and Sugihara[17] said that oxydase reaction of skin increases about fourfold in the sunshine. From the results of experiment in vitro Behring regarded the increment of peroxydase after exposure to irradiation as activation of iron in the peroxydase. Ostwald[18] reported that the increase of peroxydase reaction of the extracts from animal skin after exposure to sunlight owes to destruction of catalase. When animal is exposed to ultraviolet rays, according to Carnielli, the oxydase of the blood leucocytes augments. Katsunuma,[6] Marinesco[20] Düring[21] Makino, Goto, Kappers-Ariens[23] and others recognized the fact that the connective

tissue sheath and the endoneurium of the ending of peripheral nerve contain any oxydase and that it increases at the ending of axis cylinder but decreases near to the centripetal nerve and that it disappears entirely in the myelinated nerve. On the one hand, Masson insists that nevus cell originates from Schwannian cell, and Ito,[23] one of us, confirmed it. These facts prove the close relation between pigment production and oxydase.

According to our experiment basal cell of epidermis or nevus cell which contains a little or no melanin granules at all includes much oxydase, on the contrary, in the chromatophore or nevus cell saturated with melanin oxydase can be hardly found in it. From these facts we surmised in accordance with the achievements obtained by Katsunuma,[6] Keilin,[24] Bloch and Chodat, Szent-Györgyi,[25] Wieland, Oppenheimer[26] and others that the mechanism of intracellular oxidation is not only based upon the catalytic system of oxydase and peroxydase, but perhaps on the complicated connection of cytochrom C, iron, catalase, etc. with each other.

In the next place, according to our histochemical examination, though in endothelium of bloodvessel oxydase and peroxydase reaction are both weakly positive, but they are both strongly positive in cappillary endothelium and moreover in pericapillary tissue. Thus process of giving and receiving of oxydase between tissue cells and blood leucocytes goes on through the capillary endothelium in which blood flows slowly.

It is generally recognized that vitamin C and epinephral function are closely related to melanin metabolism. And so, this fact coincides with our result, that is, the blood peroxydase reaction is decreased by injection of vitamin C or adrenalin.

Moreover, we surmise that there is some relationship between the intensity of the blood peroxydase reaction on the guinea-pig by the injection of hydroquinone or *p*-benzoquinone—we[1] examined it having regard to the experiment on gold-fishes —and pigment production.

Besides, according to Takikawa's[27] description, iodic acid reducing substances in blood serum of vitiligo patient is much more increased as compared with that of normal case, and in the white fleck, it is increased much more than the normal skin, and although the blood glucose is almost the same as normal person but the white fleck includes slightly more blood sugar as compared with the normal skin. That is, from the increase of these two substances in white fleck, the attenuating effect upon oxydase system in that part is surmised, and it coincides with the result of our experiment in which we confirmed the attenuation of blood peroxydase reaction in the vitiliginous area and in albinism.

Many achievements about relationship between melanin production and cell nucleus were done on various animals, and though some of investigators forestall in examining it histochemically, yet they accentuate to the observations only from the direction of chromogen, thus they don't yet arrive at a definite conclusion. Having regard to our abovementioned results of oxydase and peroxydase reaction and of histochemical changes of Unna's " Sauerstofforte " and " Reduktionsorte," we think it is most necessary to investigate again from the bio-catalytic direction of view.

Summary

Oxydase and peroxydase reaction are strongly positive in cells possessed of

melanogenic facility. In case of nevus cells, these reactions are stronger in pigment-free cells and weaker in cells with much pigment. And indophenolase granules coincide with melanin granules in form and size.

In strongly reacting areas peroxydase granules are often noticed in the protoplasm, too. This means that peroxydase is transferred from the nuclei into the protoplasm, or that inhibitory power of catalase in the protoplasm is suppressed by the presence of a large amount of peroxydase.

Endothelcells of capillaries give strongly positive reaction, and the excessively produced indophenolase-or peroxydase-positive granules are disseminated in the perivascular tissue.

Oxygen-and reduction-areas are investigated by the method of Unna. In the basal layer of epidermis and the nevus cell nest of corium, oxygen area corresponds to the area where melanin to be found abundant and reduction area coincides with the area where melanin is scarce. In papillary and subpapillary layer of vitiligo, reduction area is more intense than that of normal surrounding skin.

Peroxydase reaction of local blood is more strongly positive in pigmented nevi and in the pigmented area of guinea-pig skin than in the non-pigmented part but is weaker positive in the area of vitiligo. This indicates that there are transmission of peroxydase between tissue cells and circulating myeloic leucocytes.

In the extracts of guinea-pig skin, the pigmented area shows stronger reaction than the non-pigmented.

From the above, indophenol-oxydase, peroxydase and the biochemical changes of oxygen- and reduction-areas (Unna) are surmised to participate in melanin production.

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IV. A Case of Abortive Recklinghausen's Disease*

The word Recklinghausen's disease immediately reminds everybody of the multiple cutaneous neurofibroma, but the pigmentation on the skin must be cited as an important symptome for diagnosis, that is to say, the case in which neurofibromatosis and pigmented patches developed simultaneously is the perfect form and if biassed against either of the two it is called an abortive form. Therefore, we can imagine the existence of Recklinghausen's disease which has no cutaneous neurofibromas.

We examined unexpectedly a patient who had been diagnosed the case as abortive Recklinghausen's disease and later died of typhoid fever and thus we had an oppotunity of minute examination of his whole tissue.

Clinical Examination

A 34 years old navvy was first seen on October 10, 1943. His present condition at that time was as follows; underpinhead sized black-brown clearly demarcated round multiple pigmented patches were seen disseminatedly as stars in dark night on all over the body from face to hands and legs (Plate V, Figures 19 & 20). These pigmented patches were not elevated and seemed to be multiple moles and just corresponded to the Recklinghausen's small patches, but there were neither socalled milk coffee colored Recklinghausen's large patches nor tumorlike substances over or under the skin. There were curious malformations on both of the fourth toes, namely the fourth toes were very small and parted from toe-line and seemed as if they adhered to dorsum pedis (Plate V, Figure 21); by X-ray examination the abbreviations of ossa metatarsalia quintum were found. He complained of slight exophthalmus and divergent strabismus. The blood Wassermann reaction was negative. Basal metabolic rate normal. Electrocardiography was suggestive of valvular failure. Pyelography showed remarkable narrowness of left side. Water excretion function of kidney was damaged. X-photo of the skull showed the expanding of sella turcica. Intelligence level showed a marked fall of rate. Ventriculography indicated hydrocephalus internus. Liquor cerebrospinalis presented no disorders. He was released from our hospital with above mentioned states and worked normally for full two years and died suddenly of typhoid fever in Feb. 1946.

Postmortem Examination

Date of autopsy Feb. 2, 1946. A male corpse of middling stature not very much emaciated.

Autopsy diagnosis was made by Dr. T. Kurobane: 1) typhoid ulceration from the lower ileum to the upper colon, 2) swelling of mesenteric lymph nodes, 3) bronchopneumonia, 4) leptomeningitis purulenta, 5) infectious spleen, 6) turbid

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swelling of the liver, 7) congestion of the kidney, 8) swelling of the tonsilla palatina and lingualis, 9) endocarditis verrucosa and hypertrophy of the right heartventricle, 10) lentigines disseminated on all over the body surface, 11) enlarged hypophysis with enlargement of the sella turcica, 12) under-developed suprarenal body and testis, 13) the accessory spleen of size of a walnut, 14) malformation of a toe on both sides, 15) congenital hydrocephalus internus, 16) persistence of the foramen ovale, 17) left exophthalmus, 18) funnelled chest, 19) fibrous adhesion of the right pleura, 20) ascariasis.

Among aforesaid morbid diagnosis from 1 to 9 compose the principal disease which caused the death but these have no concern with the present article so I leave them out.

Skin is generally smooth and its remarkable histological changes were as follows; first of all, at several places of epidermal cells the content of melanin increased abnormally and in those places it is found not only in basal layer but also in prickle cell layer. The little alveolar nests of nevus cell are seen in upper cutis and also pigment bearing cell among connective tissue. However I give prominence to a kind of conglomerated figure of nevus cell which is not a alveolar mass of epithelioid form nevus cell as seen in common pigmented nevi but is arranged more diffusedly or in stream lines and cellular element is prominent as the small condensed fusiform nucleus. Karyokinesis can not be proved. There are fine fibers among these cells and they are different from common collagenic fiber or Gitterfaser by van Gieson and Mallory's staining. With regard to their delicacy, paths and color tone, these are most analogous to neurinoma fiber. In some places these cells are arranged like an endothelioma but they are inclined to encircle the hair follicle, hairroot, sebaceous or sweatgland or to construct a bandlike cell bundle in these regions. Therefore, these cells can not be considered as an inflammative cell infiltration. That is to say, these cells are in a state of equilibrium against sorrounding tissue and seem to be suitable to regional structure (tissue malformation). Density of nevus cell and quantity of fiber are ununiformly; in some places accumulation of cells is prominent (Plate VI, Figure 22) and in another places fibrillisation is eminent (Plate VI, Figure 23) and we can see rarely small stratified globe-like, dactylogram-like or whirled fibrous nodules (Plate VI, Figure 24). The serial sections ascertained circumscripted proliferation of Schwannian sheath nuclei of coarse nerve bundle which was also indicated by common staining preparations (Plate VI, Figure 25). The above mentioned figures are seen all over the skin but are most distinctly on the face.

Sympathetic ganglion: Histologically, increase in stellate cells of ganglion of plexus solaris, phagocytosis of nerve cell, increasing in sheath nuclei and winding of nerve bundle are recognized and they make an impression of fore-stadium of neurofibroma. Nerve cells with two nuclei are found not rarely. There are no inflammative changes.

Cerebrum: Pia mater of facies convexa cerebri is turgid and hypertrophied and seems to be the traces of former meningitis. In general, acute or chronic changes of nerve cell and proliferation of glia cell are found. The abnormal growth of Purkinje's cell in cerebellum is found. Above mentioned hydrocephalus internus seems to have developed as a sequela of former meningitis and is not the result of recent leptomeningitis purulenta. There are no cirrhotic changes. Mongolian spot

of pia is hardly visible to the naked eye.

Hypophysis is considerably enlarged and has nearly twice the capacity of physiological one. The proliferation of parenchyme cell both in anterior and posterior lobe is found but physiological structure is kept. The anterior lobe is not a special adenoma and three kinds of gland cell are mixed almost normally. The posterior lobe shows a kind of gliosis. I can not clearly distinguish hyperplasia from benign tumor or malformation.

Suprarenal body is remarkably small and atrophy of cortex is prominent. The polynuclear slightly large cells are often seen in medula but they may be malformed sympathetic nerve cells.

Comment

In the histological study of the small pigmented patches of this abortive Recklinghausen's disease, the author observed the preneurofibromatous changes and the circumscribed proliferation of the Schwannian sheath at the subcutaneous coarse nerve bundle. And these findings are suggestive of the close correlation between the lesion in cutaneous nerve and the pigmentary disturbances, and are the evidence of my opinion[1] which maintains that the nevus cell comes from neuroectodermal origin according to Masson. Miescher and Albertini[2] classified the nevus cell into three types; Type A epidermal segregation, with groups and cords of cells, Type B infiltrative growth, exclusively intradermal and Type C neurofibromatous structure, resembling Recklinghausen's neurofibromas. Becker[3] studied 741 biopsied specimens according to this schema and stated that the type C was found in 72.5% of them. Thus the nevus cell which presents the socalled neurofibromatous structure seems to be not scarce (viz. Chapter I of this issue).

The nature of the neurofibroma of this disease has been discussed and since Verocay[4] the intimate relations to the nervous tissue have reported, for example, neurocyte (Kohn), neurogliocyte (Held), neurinoma (Cailliau)[5] endo- and perineurium (Pavisic), Schwannian sheath (Jausion, Levy, Coblentz and Spillmann[6]) and other concepts. In 1951 John and Ormea[7] investigated in neurofibromatosis the nerve esp. pathological conditons of peripheral vegetative nerve by Bielschowsky-Gros method. I have also investigated the neurofibroma by Bielschowsky-Seto's silver impregnation technique and guessed it as gliomatose peripherique.

Thus the close histogenetical correlationship between the neurofibroma and the pigmentation of Recklinghausen's disease can easily be surmised and therefore it seems to be the adequate data to my opinion that the nervous system participates to the pigment formation.

Summary

A case of Recklinghausen's disease without neurofibroma was reported. Histological findings revealed the preneurofibromatous changes in the skin and the solar plexus.

Therefore it is surmised the neurofibroma and the pigmentation in Recklinghausen's disease is close correlated in connection with nervous system.

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V. Genesis of Mongolian Spot and Blue Nevus*

In contrast with black or brown color tone of pigmented nevi, there are two kinds of congenital deep blue pigmented spots: Mongolian spot and blue nevus. The former appears generally on sacral region of newborn and fades within several years, while the latter appears anywhere but is comparatively seldom and exists for life. These two nevi resemble each other in the point that their common cardinal pathological feature is the pigment cell of deeper dermal layer. Their difference and identity have been studied at various times, although we have not come to a definite conclusion as yet.

In my former article[1] the socalled nevus cell of pigmented nevi and its origin are discussed and the neuro-ectodermal theory was accepted. In this article I intend to investigate the genesis of Mongolian spot and blue nevus by means of methods similar to that referred to in my former article; principally Bielschowsky-Seto's neurofibril staining and Bielschowsky-Maresch's Gitterfaser staining were used.

Histological Investigation of Mongolian Spot Cell and Blue Nevus Cell

Mongolian Spot: The results of investigations of the sections taken from 11 corpses of fetal six months and after, and one case of two months living body, are similar to the description given by predecessors. In other words, I indicated the Mongolian spot cell in lower half layer of cutis as fusiform, oval, stellate or somewhat elongated string-like pigment cell lying along the paths of connective tissue and with their pigment granules greater and denser than those of the basal layer of epidermis. They are so filled with pigment granules that we can hardly distinguish their nuclei. These pigment granules do not fade by hydrogen peroxide. In my examination of peroxydase in a section taken from living body by Okano C method, I found peroxydase positive granules among the black granules of melanin (Plate IV, Figure 14). This suggests that there is some relationship between this and the positive data of dopa-reaction by Bloch-Bahrawy. Then I examined closely the arrangement of these pigment bearing cells and found that although most of them exist parallel to cutaneous surface, not all coincide with the paths of connective tissues, but some come in contact with small blood vessels; some are joint with their cellular processes; some twist with blood vessels like a gabion. I can often see pigment bearing cells near small bundle of neurofibril and a little more of them at places where blood vessel and neurofibril cross each other. But the conglomeration or proliferation of pigment cells surrounding abnormally ramificating and winding nerve fiber such as found in my previous report on nevus cell, can not be seen anywhere. Moreover, no figure suggesting transition to Schwannian cell or nothing like an endoneural apparatus (fibres naeviques, lames foliacées) emphasized by Masson, is seen. The Mongolian spot cells exist next to ductus sudoriferus or can be seen around arrector muscle but are rarely seen near

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sebaceous gland and hair follicle.

On the Gitterfaser staining slides, I found the above mentioned figure of perivascular pigment bearing cells packed within argentaffin fibril and another figure closely connected to argentaffin fibril in subcutaneous adipose tissue, but with regards to Mongolian spot cells in connective tissue I could not ascertain its relationship to argentaffin fibril. Only in preparation of six months fetus, big stellate or oval pigment cells are found plentifully in the part where collagenic fiber is scarce and argentaffin fiber abundant. I could not discover in every case the inlaying figure of each cell into each reticulum, which means that the Mongolian spot cell may have some partial relationship to reticulum tissue and may be partly connected with the socalled precollagenic argentaffin fibril in its embryonal stage.

I observed no significant alterations in the epidermis in contrast to the findings of predecessors and the figures which resemble the cellules claires or Merkel-Ranvier's cell could not be found anywhere.

Blue Nevus: The first case, 18 years old female, has light blue rice grain sized nevus on her forearm; the second case, 64 years old male, has indigo blue horsebean sized nevus on his hairy head, the third case, 21 years old female has deep blue nevus on her buttock and the fourth case, 27 years old female has a half rice grain sized spot on her lower palpebra. Results of examinations of these cases are as follows: the first case has a small amount of pigment bearing cells scattered horizontally in connective tissue below the middle layer of the cutis, and in contact with blood vessels there are some fusiform and stellate ones (Plate VII, Figure 26). Therefore, we can not distinguish them from Mongolian spot cells. However, latter three cases are possessed of abundant pigment bearing cells in coincidence with the description of Tièche,[2] Sato[3] and Imschentzky.[4] These pigment cells run together with collagenic fiber in the range from the middle layer of cutis to the deeper layer (Plate VII, Figure 27) and they look like a bundle as a whole located horizontally in stratum reticulare and obliquely or vertically in subcutaneous adipose tissue. In case their black granules are dense, the neighbouring parts look like black fiber, and in case they are scarce, their nuclei are found in their centers. These pigment bearing cells are found coincidentally in or among diffused collagenic fibers. Most part of these collagenic fibers show purplish red metachromasia with Mallory's staining. This may be presumably a sort of degeneration of collagenic fibers induced by the specific biological status of tissues containing pigment cell. There are stellate, fusiform, commashaped and, rarely, ovoidal pigment bearing cells among or near fibrous pigment cell bundle (above mentioned) and their black granules are rather large. But pigment cells which have small granules show oval nuclei. Moreover, in my preparations small amount of large stellate immature cells which was found by Imschentzky in cutis of monkeys are noticed. Stellate and fusiform pigment cells are shown in contact with blood vessel or ductus sudoriferus and sweat gland. The elastic fiber disappeares almost completely in parts where fibriform pigment cells are densely located and at the layer above them.

The relationship between neurofibril bundles and pigment bearing cells studied by Bielschowsky-Seto's staining is quite different from that of nevus cells. The clearly demarcated cross-sections of neurofibrils are indicated among groups of fibrous pigment cells and there is no figure suggesting affinity between neurofibrils

and pigment cells (Plate VII, Figure 28). Moreover, that neurofibril is slightly lighter stained and has less amount of Schwannian nuclei than other neurofibril found apart from pigment cell nest. These characteristics are suggestive of embryonal unripe neurofibril. Furthermore in another preparation the pigment bearing cell groups show the enclosing and similar course to that of nerve bundle, but the said fiber has no affinity to the sorrounding fibrous pigment bearing cell just like wick and candle. (Plate VII, Figure 29). Thus the pigment bearing cell of blue nevus corresponds to endo- or perineurium and guessed to be the mensenchymal tissue.

By Bielschowsky-Maresch's staining in part where fibrous pigment bearing cells are densely located we can hardly distinguish Gitterfaser, but argentaffin fibril (stained fresh blue by Mallory's method) is easily distinguished in parts where stellate pigment cells gather together. But all of these cells are not packed within argentaffin reticulum. The stellate processes of some of these cells look like an argentaffin fibril because of their dense argentaffin granules. In the circumference of small blood vessels, however, pigment cells are packed distinctly in reticulum tissue. In this staining as well as in Seto's staining the clearly demarcated neurofibril among pigment bearing cell groups is lighter stained and an embryonic unripe neurofibril is suspected. However such belief is not conclusive in view of the labil character of silver impregnation method.

The biochemical characteristics of the pigment contained in pigment cells are as follows: ferrous reaction is negative, silver reaction positive, therefore it is assumed to belong to melanin. Faints slightly with hydrogen peroxide, especially the pigment in stellate type pigment cells has stronger resistance than that of fibrous type one and remains unchanged even by bleaching with hydrogen peroxide for one week (daily exchange of hydrogen peroxide). Thus according to the above mentioned characteristics and the fact of the simultaneous existence of dopa positive and dopa negative cells (Sato and Stranz), it seems likely that there are several stages of pigment cells of blue nevus with regards to the morphological and biochemical character and especially in connection with redoxpotential. Kreibich[5] reported the existence of lipoid in one case of blue nevus however, I was unable to confirm by Sudan III staining.

Summary of aforesaid observation is as follows: in the comparative study of the characteristics of pigment bearing cells of Mongolian spot and blue nevus, I recognized exact homology concerning the morphlogy, location, arrangement and biochemical attitudes (non-faintness with hydrogen peroxide and positive dopa reaction) of both lesions. The relationship between neurofibril and pigment bearing cell which I examined very closely, is quite different from that of nevus cells (see Chapter II). Pigment bearing cell and neurofibril do not confused with eachother as nevus cells. Thus pigment cells in both conditions coincide with each other quite well. Yamamoto[6] stated that as he found no transition between these two pathological hyperpigmentations he could not look upon these two as belonging to the same pathological entity. But my first case of blue nevus can be considered to be the evidence of histological transition and therefore, I believe that pigment cells in both two lesions belong to the homogenetical origin.

Comment

About the origin of the Mongolian spot cell, Bälz visualised the epidermal origin but the mesenchymal theory of Adachi[7] is universally accepted. Kawamura, Kiyono, Katsunuma and others advocated the histocytic theory and surmised that this is organically homologous but functionally different from the socalled chromatophores. Especially the cutaneous pigment of monkey had been minutely studied comparative-anatomically by Adachi and more recently by Imschentzky.[4] They indicated the existence of dermal melanoblast in deeper layer besides superficial socalled chromatophore, and emphasized that this is of the same species to which Mongolian spot cell belongs. The difference between these two is merely that the amount of pigment of monkey in epidermis and cutis is reciprocally proportional. In other words, when it is abundant in epidermis, it is scarce in cutis and vice versa. In a case of White female Zarfl[8] presented a jacket-like dorsoventral Mongolian spot with normal skin remaining in the surroundings of both nipples just like haloes and I observed myself a similar case to that of him. The origin of pigment cells of blue nevus has been believed to be chiefly mesenchymal since the publication of Tièche. Imschentzky studied five cases of blue nevus and found it difficult to distinguish them from dermal melanoblasts of monkeys except for the fact that in blue nevus the big stellate cell (unripe form) such as seen in monkeys is lacking. He made an interpretation that dermal melanoblast may be treated as an independent system.

As stated above, according to my observation, Mongolian spot cell and blue nevus cell belong to the same species and they are different from nevus cell and, therefore, I definitely reject the ectodermal origin, and believe that they are melanoblast of dermal origin as mentioned by Adachi and Imschentzky. However, it is not easy to decide the actual source of these cells in mesenchymal tissue. The results of Bielschowsky-Maresch's staining suggest that there exists some relationship between argentaffin fibrils and these pigment cells. But silver impregnation method is an artificial procedure and its labil character is universally known. Moreover, the uniformity of argentaffin fibril is still disputable and many new interesting findings appear one after another. So the author will not touch this point more closely and here referes only to the abnormality of congenital factor on one hand, and to the special biochemical status of the foci on the other: the former, indicated by the co-existence of pigment bearing cell and argentaffin fibrils, leads to the assumption of the remainder of socalled precollagenic argentaffin fibrils, while the latter is shown by the metachromasia of collagenic fibers in region abundant in pigment bearing cells.

Further, I cannot overlook the fact that Stranz[9] observed contemporal existence of the socalled nevus cell in 3 out of 11 cases of blue nevus. Similar mixed existence of nevus cell was observed in 1 case each by Sato,[3] Armuzzi, Dubreuilh and Petges[10] and also Komatsu in my clinic. I think that foregoing cases are the associated cases of blue nevus and pigmented soft nevus because of the frequent instances of associated symptoms in many congenital abnormalities and the fact of the pathogenetical participation of both ectoderm and mesoderm in cases of phacomatosis. And I do not think this can be regarded as the data in favor of the epidermal theory of blue nevus.

Genesis of Mongolian Spot

On the ontogenesis of Mongolian spot cells, Kato[11] observed the developmental changes of their form and arrangement, and Ishikawa[12] studied thirty-two fetal cases and established that the first sign of development is indicated already in the third fetal month and unexceptionally in all cases in the fifth fetal month.

I pursued the genetic transition of the said pigment bearing cell in one case of sixth fetal month, three cases of eighth fetal month, two cases of ninth fetal month and three cases of tenth fetal month, one case each of first and third month newborn, and obtained almost similar results to those of Kato and Ishikawa. Namely, in the preparation of sixth fetal month case (the period in which lipoblast with nucleus is proved in deeper layer) oval or pearformed pigment bearing cells are noticed in connective tissue and adipose tissue in lower half cutis, but the inclination of approaching to the blood vessel or nerve is not conspicuous. In eighth or ninth month pigment bearing cells are indicated in lower two third layer of cutis in enlarged fusiform or stellate-shapes. Moreover they approach to the nerve bundle or blood vessel, especially to the capillaries. In the fetal tenth month or newborn they are distributed abundantly throughout cutis (lower three fourth layer) except in papillary and subpapillary layer. They are distinctly stellate shaped and they communicate each other with their extended processes. At infant stage the pigment cell begins to disappear gradually at first in the lower layer, while on the other hand the figure of approaching to or enclosing nerve bundle or small blood vessel becomes manifest. In this way the said pigment bearing cell decreases with age, their cellular structure becomes indistinct and then tendency of liberation and thereupon disappearance of pigment granules is observed. Thus the pigment cell in Mongolian spot is the one with "variable" melanogenic potentiality (Masson). The Mongolian spot cell, which exists diffusively among connective tissue gradually decreases in number and only the one which is in contact with nerve fiber bundle or small blood vessel remains functionally active to the last. This phenomenon may suggest the morphological change in the development of fat cells.

As stated in the former chapter the relationship between nervous system and Mongolian spot cell is not established as yet. So the author tries to clarify this problem deductively through the histological study of blue nevus which presents similar histological picture and is properly interpreted as persistent and exaggerated manifestation of Mongolian spot. The blue nevus cell is presumed to be of mesenchymal origin corresponding with nerve sheath (peri-or endoneurium). Thus the Mongolian spot cells may have correlation to nervous system to this extent.

There is still another sort of congenital pigmentary disturbance which must be touched here in this connection. In 1939, Ota[13] designated a kind of nevus which is frequent in Japan as the Nevus fusco-caeruleus ophthalmo-maxillaris and this perhaps corresponds to the Persistent Aberrant Mongolian Spot which is reported in the United States by Cole, Hubler and Lund.[14]. In this affection, the localization coincides to the first or second branch of trigeminal nerve as shown by its designation. This fact suggests its pathogenetical correlation to nervous system. Yoshida[15] in my clinic studied histologically 24 cases of this nevus and found that the pigment bearing cell of this nevus bears close resemblance to Mongolian spot cell and blue nevus cell in its form, arrangement and in biochemical peculiarities

(non-faintness with hydrogen peroxide, positive dopa reaction, positive peroxydase reaction by Okano C method). Furthermore thread-like and fibrous pigment bearing cell of this lesion has frequent connection with nerve fiber and has equal curve and course to that of nerve and shows the intimate relationship to nervous system, so these observations become another influential and complemental data of the origin of Mongolian spot cell. It can readily be surmised that blue nevus and nevus fusco-caeruleus ophthalmo-maxillaris are the malformation consisting of abundant pigment cells which have close correlation with nervous system, thus two disorders are possessed of permanent pigment productive potentiality. On the contrary Mongolian spot loses this potentiality sooner than these two on account of gradually loosening correlation with nervous system in its process of differentiation.

Subsequently I should like to study the localization of Mongolian spot from phylogenetical standpoint. The fact that this spot occures principally on lumbosacral region and further the back of trunk reminds one of the pigment flecks in the animal kingdom. For example, Schwalbe referred to Audubon and Bachman's "The quadrupeds of North America" and noticed the darker color of dorsal area than ventral area and also Kükenthal pointed out the dark tone of vertebral area. Adachi and Imschentzky made minute topographical studies of dermal pigment spot of several species of anthropoid and surmised that the Mongolian spot belongs to the same species to that of these spots. The back of trunk corresponds to the closure surface of neural tube in fetal stage and especially lumbosacral region corresponds to the caudal rudiment of spinal cord such as the terminal of canalis centralis, filum terminale, etc., therefore it is easy to surmise the evidence of the dormants of many nervous potentialities in this area. For instance, Weidenreich, Papillault, de Costa, Ferreina and others reported the cases of lumbosacral pigmentation related to the closing failure of spinal cord and it was generally known that this area is predisposed to congenital malformations such as pigmented nevus, etc. Thus according to the localization peculiarity and the aforesaid correlation to nervous system, I guess that the Mongolian spot cell corresponds in a broad sense to the "perineurale Pigmenthülle" which exists in the closure surface of neural tube of fetus.

In 1912, Weidenreich[16] discussed the term "perineurale Pigmenthülle" in his ontogenetic and phylogenetic study of localization of pigment development in vertebrates such as anura, newt, fish, monkey and mankind. This occurs in the localization which conforms to the genesis of spinal canal and its morphological element consists generally of the stellate or thread-like cell and is indicated physiologically as the pigment bearing cells which enclose the nerve in choroid and corpus ciliare or accompany with olfactory and auditory organs (Suchanneck, v. Brunn). And also the pigmentation of human brain and pia which was described by Virchow, Müller, Gubler, Mohnike, Pfitzner, Symmers, Weidenreich and Touraine[17] corresponds to the said "perineurale Pigmenthülle" and this pigmentation is frequent in colored races. The socalled "perineurale Pigmenthülle" of Weidenreich is thought to be uniformly ectodermal origin in lower animals, while Schmidt, Kornfeld, Hueck, Borrel, Ehrmann and Takashima surmised this pigment bearing cell as the syncytial melanoblast system. Thus we have not yet the constant conclusion on the origin of the said melanoblast. But so far as the Mongolian spot cell is concerned, according to the results of Adachi or Imschentzky of anthropoid and monkey and my

previous and above-mentioned descriptions, I am convinced of the origin from the mesenchymal melanoblast and conjecture that this indicates the arrangement of a kind of " perineurale Pigmenthülle."

Thus I conclude the pigment bearing cells of Mongolian spot, blue nevus and nevus fusco-caeruleus ophthalmo-maxillaris are dermal melanoblast with same evolution mechanism. And the evidence that the physiological factor viz. Mongolian spot disappeares in a prescribed duration corresponds phylogenetically to the partial regressive alteration of inguinal apocrine gland in mankind and also to the widespread and protracted existences of dermal melanoblast and apocrine gland of monkey and other hairy animals. On the contrary, blue nevus and nevus fusco-caeruleus ophthalmo-maxillaris are interpreted as localized malformation which exists for a long time like the aberrant teratoma of apocrine gland.

Inheritance of Mongolian Spot

At first Bälz advocated the racial theory about the genesis of Mongolian spot, but subsequently this affection was indicated histologically as the same pigment bearing cell on lumbosacral skin in all races of the world unexceptionally even in White races and recognized as the human physiological character (Adachi, Bloch and Bahrawy), therefore Bälz's theory was absolutely neglected. Also the mutation theory of Apert and the atavism theory of Ikeda, Darwin, Bruch and others can not be accepted from the same reason in view of recent genetics.

But the distinct racial difference in degree and frequency of development of these spots is undeniable, *i.e.*, very low percentage in the Aino race even in Japan and also the investigation results of Sperk and Koòs in Hungarian mixed breed races were relatively complicated. In the observation of the mulatto Bülow[18] reported more frequent development of Mongolian spot in the back cross (F_2) than in mulatto (F_1) between White and Samoan. Ferreina[19] observed sixty-three mulattoes in Brazil and reported the frequency of fourty three per cent in Black and five per cent in White. In Peru Eyzaguirre[20] reported frequent development in the mountain Indians (pure line), but low percentage in coast territories perhaps because of many crosses in these territories. After all it is certain that the spot develops most frequently in colored pure lines. Mayerhofer[21] made minute study according to Zarnik's Rassentafel, and noticed at last the inclination of frequent development in the race which shows dark color of skin, hair and iris such as the Dinarierian in Europe and also he recognized the high incidence in dark colored individual, and he reached to the socalled pigment theory.

However, it is certain without dispute that these problems must be discussed by all means in the field of genetics because the body-color of mankind depends upon the race to which he belongs, therefore further observation must point to investigate its degree, arrangement, etc. by geneological or twin examinations. But such reports are scant and at best I find few cases in Europe such as brother and sister cases of Fujisawa, Tugendreich, Koòs, Cozzolino, Comby *et al.* and the twin case of Silber and the triplet case of Orel and the Japanese who has this spot almost in hundred per cent takes no account of this problem. Therefore it seems difficult to investigate the problem by these genetical-statistical method and here I must appeal for complemental data within the phylogenetic investigation. It is already

admitted that the interaction of genes plays an important rôle in the inheritance of body color or pigment spots in animal through the following results of investigation, *i.e.* in Durham's hair-color experiment of mouse, he indicated the complementary color factor of black and white; the inhibitor theory of the cocoon-color of silkworn and the feather-color of chicken ; also the conditional factor of hair-color of rabbit. Especially Castle's observation upon the modifying factor of Dutch rabbit is full suggestion in the localization and size of pigment spot. Moreover recent biological investigations suggest that the formation of pigment depends upon the two factors; melanogenic substance and oxydase. And Wright assumed the existence of two enzymes of hair-color of animals and now the physiologic-genetical interpretation is influencial.

For a long time racial crosses being present in mankind and therefore the observation upon pure races is not easy and it is difficult to comment on the genetic problem of mulatto by demographic method, so the color of skin, hair, eye, etc. of mulatto being suspected for long time of blended inheritance. But since Davenport, Fischer, Hurst, Lotsy *et al.* indicated the cases of segregation in their studies of back crosses between White and Black the evidence of polymery such as in animal being gradually suggested.

The Mongolian spot is indicated in all races with difference of phenotype as mentioned above and it must be treated in genetical standpoint as a physiological character similar to animal or human skin-color. And it is proper that interpretation according to the polymeric inheritance should gather strength. But hitherto the observation of these manner is poor and I cite here only the contribution of Larsen and Godfrey.[22] Namely both investigated and tabulated seven hundred cases of Mongolian spot in Hawaii and discussed by means of two factors; *i.e.* the factor for pigment (P-dominant) and the factor for presence of mark (o-recessive) and recently Maxia[23] made a geneological observation in Sardinia and surmised that the Mongolian spot is inherited as polygenic dominant. I think these theories seem to be reliable.

I cannot observe these evidences because I belong to the race which shows the Mongolian spot in hundred per cent thus I guess at the ploymeric inheritance (which corresponds to the qualitative factors) according to the above mentioned experimental genetic observation and to the report of Larsen and Godfrey or Maxia and also to the point that this spot is indicated histologically in all human-beings.

Summary

I confirm that the pigment bearing cell of blue nevus corresponds histologically to the endo- or perineurium of mesodermal nature. Therefore I guess that the Mongolian spot cell originates from the same origin as blue nevus and that it is the mesodermal melanoblast which corresponds to Weidenreich's socalled " perineurale Pigmenthülle."

And I agree to the polymeric inheritance theory of Mongolian spot according to the physiologic-genetical considerations.

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VI. Angioneurotic Reaction of Mongolian Spot*

The correlation between cutaneous pigment and nervous system has been studied for many years and various experiments were attempted upon many species of animals and especially Kudo, Schaefer,[1] Hewer, Hemmeter, v. Frish *et al.* reported the darkening of fish skin following the amputation and destruction of sympathetic nerve at certain portion and also it was known that the irritation of peripheral end of that nerve results in the relightening. The factors which indirectly react on the melanophore and cause its contraction or dilatation are the alteration of moisture, atmospheric pressure, temperature, and moreover the participation of chemical substances was also studied as follows. Gianferrari, Spaeth and Hewer, Hemmeter, Hogben[2] observed the contraction of fish melanophore by adrenalin and in our department Itô and Nakajo[3] proved same results on Japanese black goldfish *Kurodemekin*. Lieben reported the constrictive action of adrenalin against the melanophore of frog web without the alteration of blood flow. About caffeine, muscarine, atropine, strychinine, ergotamine, cocaine the constant effect has not been obtained but Gilson reported that the small dose of narcotica and large dose of alkaloid such as cocaine, physostigmine and atropine dilates the melanophore and small dose of physostigmine and cocaine causes the constriction of melanophore. Kahn reported the darkening with pilocarpine and nicotine. Fuchs observed darkening with nicotine.

The correlation between human cutaneous pigment and nervous system has been studied on both increase and decrease of pigment. Jadassohn observed a case of genital leucoderma in tabes dorsalis and the anomalies of cutaneous pigment in lepra nervosa, the segmental arrangement in vitiligo and the pigment anomalies which accompany with sweat secretory disturbances are already known. Thus the coordination of vegetative nerve to cutaneous pigment is easy to be surmised. Similar observations were performed on nevi esp. on nevus linearis, and Voigt's line, Eichhorst's curve, hair stream and dermatom have been discussed. But it seems difficult to arrive at a definite conclusion because of reasons such as complexed peripheral innervation of skin, interference of vegetative and sensible nerve, individual variation and the difficulties in histological and physiological investigations of cutaneous nerves. In this supplement Itô[4] stated that the pigment cell of Mongolian spot corresponds to the endo- or perineurium and now I made the clinical examinations and histamine scratch tests on newborns, infants or children with Mongolian spot.

Clinical Observation

Age of patient: From one month newborns to six years old child.

Sex: thirty males and twenty five females.

Site of spots: thirty three cases have the spots only on sacral region and other cases not only on sacral region but on other region (seventeen on back, one on ab-

* By Yoshiro Hamada.

domen, one on upper extremities, three on lower extremities). In all cases I did not observe any spot on the breast but Zarfl[5] reported a case of four years old female with Mongolian spots on navel, hypogastrium and perimammilar region besides on nipples. And I formerly observed myself a similar case to that of Zarfl in one year old male infant, who had a jacket form dorsoventral spot with physiologic skin remaining surrounding both nipples just like haloes. I believe that these findings resemble the characteristic feature of qualitative and quantitative arrangements of nipple innervation which was studied by Suga[6] at the anatomical laboratory of our university.

Hairiness: Schohl, Zarfl and Kerl indicated the hairiness cases and I observed that in 3 cases.

Number of spots: from one to seventeen.

Symmetry: The symmetry was observed in 3 cases. One of which is a male infant aged seven months having a palm sized deep blue spot on gluteal and lumbal region and its upper border coincides with Jacobian line and shows almost perfect bilaleral symmetry against the vertebra.

Size: from rice grain sized to three times the size of a palm.

Color: light blue, ephelide-like, sooty, mole-like, tattoo-like and others. A spot is generally darker when it exists on lumbosacral region.

Boundary: The clearness or undistinct border of spot does not depend upon the color gradation and the elevation of spot is not observed.

The correlationship between Mongolian spot and malformation: Epstein, Bruch, de Costa emphasized this and Yamamoto also reported that the malformation may be found by a minute examination but so far I have observed no malformed cases.

The correlation between Mongolian spot and other dermatoses: I observed one case of two years old female of strophulus infantum but no eruption on Mongolian spot and three cases which have intertrigo and other eczema on gluteal or genitocrural regions with no eruption on their Mongolian spots.

Histamine Scratch Test

The histamine test has been performed upon many dermatoses such as leprosy, vitiligo, alopecia areata, scleroderma, etc. and Yamada[7] also tested in angioma and he stated about the pathogenetic mechanism of this affection. However, data on Mongolian spot are quite phenomenal, therefore I performed the following test on Mongolian spot.

Method: The test consists of linear scratching with a needle on both normal

TABLE VI

Type	Development of wheal on Mongolian spot	Cases
A	No wheal	3
B	Later than on normal skin	18
C	Simultaneously with normal skin	7
D	Sooner than on normal skin	1
E	Elongates on the extension of scratched line	2
		Total 31

skin and Mongolian spot and instant application of 1 :1000 histamine chloride aqueous solution on the scratched line. The time required for the development of erythema and wheal is measured.

Type A. Mongolian spot shows no wheal.

Case 1.—A male, aged seven months has an oval spot on his right buttock, of which long axis is vertical to vertebra and light blue gooseegg sized spot lies partially upon this spot with a darker spot in the center. He has also two spots on the right back.

Case 2.—A female, aged nine months has five spots on the buttock (two of which are deep blue) two on the loin, six on the back, three on the light upper arm and one on right patella.

Case 3.—A female, aged two months has a spot on the coccygeal region. The right half portion of this spot is deep blue.

These three cases show no wheal.

Type B. Wheal develops later on Mongolian spot than on normal skin.

This type consists of eighteen cases. Differences based upon the site, hairiness, color and size of spot are not taken into consideration. In general it seems that this type belongs often to the multiple Mongolian spot. The interval of wheal development on normal skin and Mongolian spot varies from eighteen seconds to ninety seconds. The attitude of the wheal on Mongolian spot which developed following that of normal skin is of interest. In a six years old male, histamine wheal desappears after five minutes just like as one erases something written on a black-board. And in a female aged three years four months with a palmsized bilateral symmetrical spot on her gluteal region the histamine wheal developed at thirty four seconds but at three and half minutes the wheal on the spot is distinctly narrower and lower than that of normal skin. No erythema exists on spot but in normal skin the diffused erythema was found all the time. I also recognized the elongation of wheal on the extension of scratched line (see type E).

Type C. Wheal on Mongolian spot develops simultaneously with normal skin.

The interval of wheal development is varied. The wheal develops in twenty five seconds on average. In the minimum case erythema develops in eight seconds and in the maximum case erythema develops in fourty eight seconds and extends diffusedly along a scratched line.

Type D. Wheal develops on Mongolian spot sooner than on normal skin.

In a male infant aged two months, the wheal develops on Mongolian spot after fifteen seconds and on normal skin it takes thirty five seconds.

Type E. Wheal on Mongolian spot elongates along the extension of scratched line.

Case 1.—Male, 1½ years old, the wheal on Mongolian spot develops simultaneously with normal skin but it elongates towards its center along the extension of scratched line.

Case 2.—A male, aged seven months has four spots on buttock, one on right shoulder. On normal skin erythema develops in twenty eight seconds and disappears after five minutes. After six minutes wheal on Mongolian spot elongates towards the center of spot on the extension of scratched line. In this case two linear scratch line were made parallel on both normal skin and Mongolian spot and contiguous wheal which developed

on both of the two scratched lines shows intensive inclination to be fused in Mongolian spot area than in normal skin.

Summary

The author examined fifty cases of Mongolian spots and the histamine tests were made on thirty one cases.

The histamine wheal on Mongolian spots seems to differ from that of normal skin as regards its development, degree, age of patients, etc. as follows: seven cases show the simultaneous wheal development on both normal skin and spot, in one case the wheal on the Mongolian spot develops sooner than on normal skin and in other twenty cases it developed later than on normal skin but three cases showed no wheal on Mongolian spot. The distinct difference of wheal on the border of normal skin and Mongolian spot was observed in two cases, six years old male and three years four months female.

Thus the difference of angioneurotic reaction between Mongolian spot and normal skin can be surmised. Moreover the fact that the several cases in which the reactive dermatoses such as strophulus infantum or eczema did not attack the area of Mongolian spot seems to be remarkable in that respect.

Therefore I think that the Mongolian spot cell which corresponds to the mesodermal pigment cell has some correlation to nervous system.

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VII. Nevus Fusco-caeruleus Ophthalmo-maxillaris Ota*

The nature of mole, pigmented nevus, blue nevus and Mongolian spot which belong to congenital melanosis had early been discussed and their pathogenesis had generally been made clear. But in 1939 Ota[1] took notice of one special form among socalled pigmented nevi found especially on face and named it " Nevus fusco-caeruleus opthalmo-maxillaris Ota." Tanino[2] stated its detailed clinical observation; in general, its typical case is unilateral, occupies the cutaneous distribution of the first or second branch of trigeminal nerve and is arranged diffusely as grouped ephelidelike spots in blue, brown and their middle tones and is often accompanied with melanosis bulbi. The origin of the pigment bearing cell, however, is not yet sufficiently explained and the recent supplemental reports in Japan limited to the casuistics. On the other hand, in the United States the differences between the similar cases to this nevus and blue nevus, Mongolian spot or argyria have been discussed. But its origin does not come to be investigated thoroughly at all. Therefore in the series of 110 cases of this disease at our department, I state first the clinical observations and in the second part present the histological and histochemical investigation.

Observation of Author's Own Cases

Before describing author's own cases I summarize Tanino's classification of his 26 cases; he classified as follows; 1) mild-, 2) moderate-, 3) intensive- and 4) bilateral-type. The mild orbital type is faint, the arrangement of pigmented spots is scattered and they are distributed over upper and lower palpebra, periocular and temple regions; mild zygomatic type is found between sulcus infrapalpebralis and sulcus nasolabialis and over zygomatic region. Moderate type distibutes over the upper and lower palpebra, periocular, zygomatic region, cheek and temple, and about half of it invades radix and ala nasi also; intensive type spreads over besides above mentioned parts even to scalp (parietal and frontal region), forehead ,brow, radix, ala and apex nasi; and bilateral type is the case in which abovementioned types are found on both sides. However, Sakurane[3] reported the additional cases in which the pigmentation reaches to the distribution area of the third branch of trigeminal nerve (n. mandibularis), or to that of cervical or thoracic nerves. On the other hand, there is a type which is seen only at the apex and ala nasi or in the nostril.

I observed 106 cases among 110 in amount except 4 cases which were insufficient in clinical description. *Frequency:* From 1940 to 1950 the patients of this nevus amounted to 110 cases and all the dermatologic out-patients in this period were 27082, and its rate comes to 4.06% and this is 21.7% for congenital pigmented spot which appears on face.

Sex: Among 106 cases 22 were males and 84 were females. The decided

* By Kunio Yoshida.

predominance of female, it appears, perhaps depends on the cosmetic complaint.

Type: Among 106 cases 45 were of mild orbital type, 22 were of mild zygomatic type, 19 were of moderate type, 10 were of intensive type (Plate VIII, Figure 30) 4 were of bilateral type, 3 were of each abortive and perinostril type, in brief mild type amounted to 2/3 of all cases.

Location: The lesion was most found at upper and lower palpebra and next found on the temple, zygomatic region, cheek, forehead, etc. In comparison with the cutaneous innervation, 18 cases were found only in the 1st branch region (n. ophthalmicus), 27 in the 2nd branch region (n. maxillaris), 55 extended to the both regions, and 1 extended to the whole region from 1st to the 3rd branch, 1 extended widely to the 3rd branch, the 3rd and 4th cervical nerve and the 2nd thoracic nerve, and 3 were only found at the apex, ala nasi and nostril. By the way, in 1 case which attacked both 2nd and 3rd branch regions pale blue pigmentation was also found on the buccal mucous membrane.

Side of lesion: 61 cases were on the right side, 39 on the left side and 6 cases were bilateral type. In comparing with type of lesion the difference between right and left side was scarcely found in the orbital type among mild type, but zygomatic moderate and intensive type are frequently found on the right side. From this it may be thought that there is any participation in the intrauterine position of fetus, but there is no significant difference between the 1st and 2nd attitude in the Japanese, so the factor which controls the side of lesion is not understood at all.

Age of onset: In 65 cases the lesion appeared by birth or soon after birth and 41 cases were the delayed-type. About half cases among them were mild orbital type, so it seems that there were many one who found the nevus late, and 4 cases in which one could not distinguish the nevus without the attention because it became considerable in each puberty, 1 case in which the pigmented nevus became remarkable after marriage, 6 cases in which the conditions such as contusion or collision, etc. motived the nevus. Thus I can't deny the fact that the embryonal terrain which grows very slowly is promoted or strengthened under the physical disharmony or some other inducement.

Age of consultation: Like common nevus many consult in infant and in puberty and patients from 1 to 2 years amounted to 21 cases, from 14 to 20 years 28, from 21 to 25 years 32, from 26 to 30 years 9, from 31 to 40 years 8 and from 3 to 13 years only 4 cases. According to the types of lesion patients who consulted in 1 or 2 years of age were 31.02% in moderate and intensive cases, while in mild cases they amounted to almost half, that is 16.4% and it seems natural on account of the cosmetic complaint that many patients of intensive type consult early in infancy.

Color tone: Color tone was generally spotted color which consists of ephelide-like brown and blue-nevuslike blue, and showed many tones of color, that is to say, black, blackpurple, blue black, deep blue, blue, fading blue, slate-blue, purplish brown, brown, and faint brown. This seems perhaps to depend on the healthy complexion and the quantity or the depth of pigmentation. The boundary was faint on account of fading in the periphery. These color tones scarcely changed after its perfection, but especially in female changes of the color tones were more or less seen according to the conditions such as menstruation or others. The observation of these changes was as follows; in 10 cases the color tone of whom became dark

during menstruation, 2 were the cases of fading, 2 were those of darkening when they became fatigued, each 1 case of seasonal darkening in summer and winter, and 3 cases showed the diurnal changes of color tone. By the way, this nevus was often found in one whose skin-color of face was darker for Japanese, and seldom in the women who had such snow-white complexion as seen in Tohoku district.

Melanosis bulbi: It was observed in 65 cases (61.3%) among the whole cases. Melanosis bulbi was found in the whole case in the intensive type, in moderate type 13 were found among 19, in the orbital type 28 among 45, that is, it is found in 2/3 cases, but in zygomatic type it was found only in 8 cases among 21. This melanosis bulbi was almost unilateral and was observed in accordance with the side of nevus, but in 6 cases found on both sides, and was only found on both sides in 1 case among the bilateral 4 cases, 2 cases was only found on the side high in the degree of pigmentation, and was not found in 1 case at all. The extent of melanosis bulbi varied between those in which the frontal surface of eye-bulb was almost blue in color to those in which the pigmentation was millet grain in size, while the color tone was also various from blue-black to brown and in the widely extended cases it is dark in blue and it becomes brown in proportion to the extent of spot.

Inheritance: In the observation of 106 cases, in 1 case each his mother and his cousin were also affected but the clear inheritance was not found. There was a patient whose mother had the blue nevus which is analogous disease to it and 14 cases in which the pigmented nevus was found in parents, children, brother and sister or relatives. There were two patients whose parents were consanguinously married.

Histamine scratch test: I determined the onset of erythema and wheal of 14 cases after linear scratching and instant application of 0.1% histamine chloride solution on both the nevus and healthy skin (see Table VII), 8 cases appeared earlier 4 simultaneously and 2 later on the nevus than the healthy skin and therefore the angioneurtotic disturbance of the nevus was observed in about half cases.

TABLE VII

Sex	Age	Erythema	Wheal
F	24	+30	+90
F	22	+20	+90
M	2	+20	+90
F	17	+10	+60
F	32	+10	+30
F	23	0	+30
F	21	0	+20
F	16	0	+10
F	23	+ 5	0
F	18	0	0
F	4	0	0
F	15	+10	0
F	25	0	−30
F	29	0	−30

Values in the table show the interval of erythema or wheal development on the nevus in seconds. (+)...sooner than, (−)...later than and (0)... simultaneous with erythema or wheal development on the normal skin.

Diaphoretic test: There was each one case in which the diaphoretic time of the nevus by the method of Wada[4] was faster or later than the healthy skin, and I will hereafter pursuit to investigate.

Treatment: Like the pigmented nevus, the carbone dioxide snow, rongalit

C or albalite C, were often applied, but in cases of highly developed lesion and being deep in blue color tone this treatment attacks the deeper layer of the nevus and leaves leucoderma or cicatrice, etc., and therefore it is difficult of cosmetic effect. I examined the alternative continual injection of vitamine C, adrenalin and adrenocortical hormone in turn in 15 cases and observed considerable effect in 6 cases, but in part of deep color tone the specially remarkable fading was not observed.

Histological and Histochemical Observations

24 out of 110 author's own cases were biopsied. Most specific alteration in the histological picture was seen in the corium; like Mongolian spot the pigment cells were seen most in upper reticular layer, next in middle layer and recognized in lower layer or about adipose tissue, and their sizes were various; the cells in comparatively upper layer are fusiform or stellate in shape and most of them became long string- or fiber-like cell as they go down to lower layer (Plate VIII, Figure 31). These very large cells became gradually slender in the periphery, and dendritic type cells were connecting with each other. Especially the pigment cells which were seen in adipose tissue were communicated with each other as if they embrace it. These pigment cells were seen sporadiacally in the cutis and some of them coming together, form such a bundle as was seen in blue nevus. Generally speaking, the long axis of the pigment cell was often parallel to the cutaneous surface and it was nearly accordant with the course of connective tissue, and intervened among fibers. But it arranged obliquely or vertically in the cells around small blood vessel, around sweat gland, sweat duct, sebaceous gland or among adipose tissue. The pigment granule which is the refractional melanin granule packed in these cells was much coarse in comparison to those of basal cell layer, and was so full in fusiform or stellate cell of upper layer that the identification of the nucleus was difficult; but the pigment granule was comparatively less in long and slender stringlike cell, in some cells it was only seen near the center, and the long elipsoid nucleus could be found. Besides, in regard to the alteration in corium, the slight perivascular round cell infiltration was found in reticular layer, but it was comparative distinctly demarcated with the circumference and this gives different feeling from the common inflammatory cell infiltration. Such nevus cell as in soft nevi was not found in any cases at all. Though there were differences in degree, in all cases chromatophore was recognized in small number in papillary and subpapillary layer.

In epidermis, the alteration was scarcely found, and the following fact was only acknowledged; the vacuolization of prickle cell, a few clear cell, or the slight spongious alteration, but there were no typical one. Though in some cases the arrangement of basal cell layer was abnormal, in general it was almost regular, the content of pigment was more or less irregular and in some part it increased even to the middle of prickle cell layer. While in some other part it was very little found in basal cell layer. The extent of increase and decrease of pigment is limited to several epidermal ridges, and thus the correlation was not admitted between the quantity and the arrangement of pigment cell in the abovementioned corium and that of epidermis.

By the way in consequence of inquiring the quantity of pigment cell, the degree of depth and the correlation between the content of pigment and the clinical color-tone in epidermis, it is considered rather natural that the blue tone is deep in those

cases in which the pigment cells were deep situated, and on the contrary the brown tone at the inspection is found considerably in those cases in which pigment cell was found only in the upper part of reticulum layer and pigment granule was abundant in basal and prickle cell layer.

Neurofibrille staining: The existence of nerve fiber which might connect with pigment cell was investigated in many specimens according to Bielschowsky-Seto's method, and I found that in few specimens nerve fiber bundles or nerve fibers ran near the pigment cell or with course of it which intervened in the connective tissue; long and slender stringlike fibrous pigment cells were close together and ran with the same bend to that of nerve fiber (Plate VIII, Figure 32), but transitions of Schwannian nuclei as in pigmented nevus were nowhere found at all. These observations resemble closely to what Ito[14] pointed out in blue nevus. But the figure, the middle axis of which was nerve fiber, was not found because the nerve fiber bundles of the pigment cells of this nevus were not so remarkable as blue nevus.

Histochemical Examination. *Dopa Reaction:* According to Fujiwara's[5] experiment under the titel of the "Influence of formalin upon the dopa reaction" dopa reaction becomes gradually feeble in the formalin fixation, but the fixation for 20 to 30 minutes does not influence the result at all. Thus the excised specimens were fixed in 10% neutral formalin for 30 minutes and were cutted in frozen section. The positive reaction was recognized in the specimen which was immersed in dopa solution for two hours. This reaction is not always positive in all pigment cells but it is positive in some of stringlike pigment cell, in which smaller black granule than melanin granule collected especially at the margin of the cell (Plate IV, Figure 15 and Plate VIII, Figure 33). The dendritic melanoblast in basal cell layer was distinct and leucocyte was stained strong positive.

Oxydase reaction: The fixed oxydase reaction was studied by the method of T. Ogata. This reaction which shows the blue granule in the protoplasm of myeloic leucocyte, was negative in the pigment cell of this nevus which was full of pigment granule, but in the stringlike cell of deep layer the positive reaction was found which had blue granule which did not refract and was nearly as large as or slightly smaller than melanin granule. Moreover, the petty granules were seen at or around cavity of blood capillaries and in some cases slight positive granules were recognized at the part near nervefiber bundle.

Peroxydase reaction: According to Okano C method, all nuclei were stained blue without discrimination and in the cells of epidermis and external sheath of hair root blue granules being accordant with chromatin were noticed. Moreover the intracellular granules were found in the part near the basal layer. In regard to pigment cells which were abundant in pigment granules the results were same as the beforementioned oxydase reaction, that is, negative or difficult to distinguish. And the intracellular granules stained in blue were seen in the stringlike cells being short of melanin granules.

The chromatophore was sometimes remarkably positive but in many cases negative. In the specimen which was fixed in alcohol and, embeded in celloidin and stained by Unna's method after cuprum sulfuricum procedure in the method of Sato and Sekiya, minute blue granules in protoplasm were found more distinctly in the cells being short of melanin granules than by Okano C method.

Reductive reaction by silver impregnation method: Epidermal pigment which is indistinct in hematoxylin—eosin staining is indicated most evidently as the black granules even in rete Malpighii by Bielschowsky-Seto's method. In upper reticular layer so much reduced silver granules are found in the cells which have abundant pigment granules that each of silver granules could not be distinguished, but in lower layer these granules are found as the dark-purple black granules in string-or fiver-like pigment cells, and it is learned that intensive silver reduction is found at the oxygen area of Unna (see below).

The investigation of oxygen- and reduction-area by Unna's permanganate-methylgreen method: I did not recognize the particular alteration in reduction area such as horny layer, hair follicle, but in pigment cell the oxygen area which stained bluish with methylgreen was recognized (Plate IV, Figure 16).

Hydrogen peroxide procedure: The frozen section was immersed in 3% hydrogen peroxide aqueous solution and the pigment of basal cell layer showed remarkable fading after 24 to 48 hours, but there was no fading in the chromatophores of papillary and subpapillary layer, and the pigment cell in corium began to fade after 120–144 hours. The resistance to fading was stronger in stellate pigment cell than stringlike one.

The influence of adrenalin: In the specimen which was excised after local intracutaneous injection of adrenalin, pigment cells were seen distinctly because of conglomeration of each melanin granules but the cells themselves appeared somewhat constricted. I do not think that the temporary fading following local intracutaneous injection which was mentioned in the clinical observation owes only to the constriction of blood vessel.

Meirowsky's and Bittorf's phenomena: In the observation of the fresh specimen which was suspended over saline and incubated at 56° C. according to Meirowsky[6], already after an hour, the pigmentation of epidermis and then that of corium became remarkable after four hours, and after 24 hours the blackning finished completely and attached hairs increased the degree of blackning too. The skin specimen which was immersed in the solution of 1:1000 adrenalin at 37° C. (Bittorf's method[7]) showed the mild blackning of epidermis after 24 hours.

Fat staining: Kreibich[8] indicated the lipoid in the pigment cells of blue nevus. I tried Sudan III staining, but I could not recognize the positive reaction in the pigment cells.

Comment

Review of literatures: This disease had originally been considered as the pigmented nevus and was especially taken notice since the report of Ota and Tanino and afterwards Sakurane, Niisawa, Hanada, Nomura and Nagao reported each one case, and Hanahata and others reported 2 cases of Japanese, 5 cases of Korean, Ohara each one case of Japanese and Korean, and S. Kitamura 5 cases, Kan 2 cases of Korean, and in Manchukuo and China some cases are reported.

In the European and American literatures, however, only in the ophthalmologic reports of about 10 cases could be found as the case of melanosis bulbi accompanied with cutaneous spot but the case report as the cutaneous disease is seldom found. What Goldschlag[9] reported as the blue nevus seems to be this disease from the point of cu-

taneous spot and the pigmentation of eye-ball. In the United States the reports are made recently; in 1931 Ebert and Nomland reported the case of 23 years old female whose pigmented nevus of the sclera had became distinct 8 years before and the pigmentation of the left temple appeared 3 years before, in 1935 O'Leary, Montgomery, and Brunsting reported a case of 25 years old female as the metallic pigmentation, in 1936 Rauschkorb that of 22 years old female, it 1937 Cornell remarked the pigmenttation on left face of 28 years old female, Mitchell and Scull reported the case of 47 years old Negro, in 1938 Jamieson that of 21 years old white female, Schiller and others that of 12 years old male, in 1939 Montgomery that of 17 years old female and in 1948 Rothman and others reported the case of 20 years old racial mixed female between Japanese and American. All those were casuistics at the society or discussion under the suspicion of blue nevus, Mongolian spot or argyria, and those natures were not settled, but except for the case of Schiller in all cases the borne or delayed pigmentation of the sclera was found, and it seems to be this nevus in view of the site of lesion. While in 1949 Pariser and Beermann[10] advocated the morphological variant of blue nevus, the persistent extrasacral Mongolian spot, or the diffuse mesodermal pigmentation under the title of the wide blue-spot-like pigmentation in a case of 34 years old white male, in 1950 Cole, Hubler and Lund[11] described minutely 4 patients as the persistent, aberrant Mongolian spot, and Mereer[12] remarked in the discussion that these pigmented nevi were called the nevus fuscocaeruleus in Japan, and these 3 out of 4 cases in accord with the case of Pariser are just undoubtendly this disease according to the description of eye-bulb, skin lesion and their histology. That is, in the United States this disease has been comparativery well observed and reported but it belongs originally to the rare disease. In conclusion, it may be said that this nevus develops without regard to race, but from its frequency it can't be denied that the Japanese who has Mongolian spot at the rate of 100 per cent is predisposed to this nevus. It may be guessed, therefore, that this is the reason before Ota's report why the brown case was considered as the pigmented nevus and the case which has the blue tone is supposed as the aberrant Mongolian spot or the variety of blue nevus.

It is a very interesting problem why the nevus is found on the region of the first or second branch of trigeminal nerve, but the explication is very difficult and can't be easily settled. That the pigmented nevus, hemangioma, etc. often develop on those parts has been generally recognized and from the figure of genetical observation which Castle incidently made in Dutch rabbit, the variations in the extent of pigmented spot by the action of modifying factor are noticed and especially the peripheral zone of eye seems to be the predisposing area of pigment spot. After all, of this pigmented spot too the following guess is only made; this pigment spot will appear on face by the abovementioned promoting factors in addition to the complex embryonic conditions.

Melanosis bulbi is the second feature of this nevus and Bourquin[13] designated as the complete melanosis which has the three cardinal symptoms; partial pigmented spot of sclera, hyperpigmentation of iris and the dark brown colouring of fundus oculi, but some of these three may be found not only in this nevus, but in hemangioma on face especially near eyes, pigmented nevus or verrucous pigmented nevus, etc.. However, many are incomplete in Bourquin's sense in those cases

and it is only in this nevus that complete melanosis bulbi in the sense of Bourquin is seen and this can be said one feature of this nevus.

Moreover, that in many cases erythema and wheal which appeared fast on skin in the histamine scratch test showed angioneurotic hypersensivity of the lesion, and I think that this disturbance may have at little concern with the pigment formation of this nevus. According to the facts that the contemporal fading is observed after 5–30minutes of local intracutaneous injection of adrenalin, that this nevus appeared or became remarkable in puberty, and that alterantion of color tones was recognized in female patients in the physical changes such as menstruation or others, I guess that the pigment formation depends on the neuro-hormonal reaction.

The histological and histochemical investigation of this disease is rare, and in Japan only Tanino mentioned the histological observation of two cases; in the United States Cole and others investigated four cases, Rothman & Pinne, Mitchell & Scull, O'Leary, Montgomery & Brunsting, Ebert & Nomland made the biopsy in each one case and discussed particularly the difference with argyria and considered the pigment granule as melanin, and Cole and others, and Rothman & Pinne obtained positive dopa reaction in their cases and they stated that the pigment cell was the melanoblast and that it would be the abortive form of the aberrant persistent Mongolian spot or blue nevus.

From the results of the histological and histochemical observations which I investigated, I think that this nevus is almost similar to blue nevus or Mongolian spot; Tièche proved in the blue nevus that the pigment cells groups which pack the brown pigment granules—melanin in the cells take of various forms in connective tissue fiber at the middle layer of corium, and Sato and Stranz verified the positive dopa reaction in that pigment cell. Bloch-Bahrawy demonstrated the fact that also in Mongolian spot the oval, fusiform, stellate or dendritic pigment cells in connective tissue fiber are found and dopa reaction of these cells is positive. Ito[14] scrutinized the abovementioned two diseases and asserted that they were the mesodermal melanoblast. Now, in the nevus fusco-caeruleus ophthalmo-maxillaris, pigment cells of various forms lay scattered in corium and the nevus cells could not be found and the pigment cells kept its color tone firmly without fading with hydrogen peroxide for 6 days. Sato and Stranz pointed out that the coexistence of the negative and positive dopa reaction in blue nevus and that there were many degrees of oxidation; my observation on this nevus was almost accordant with them and the dopa positive granules were proved in cells being scant of melanin granules, and the reaction became negative in the cell abundant in pigment granules—melanin, therefore I guess that the cell having dopa oxydase in excess perhaps becomes dopa positive in compliance with the alteration in the process of oxidation and it is surmised consequently that they were due to the same origin from the piont of the chemical nature of the pigment cell.

The remarkable oxygen-area which was revealed with methyl-green in pigment cells was recognized by Unna's method and the positive oxydase and peroxydase reactions which showed minute blue granules in the cell being short of pigment granules were noticed. These findings coincide with the following histochemical observations on pigmented nevus of Itô and Watanabe.[15] They verified the participation of oxydase and peroxydase in melanin production and said that

peroxydase existed in excess in nucleus moved to protoplasm, overcame the catalase action and participated in the oxidation of melanogen and that remarkable oxydase reaction was found in the state of hyperfunction of melanogenesis. From abovementioned facts the pigment cell in nevus fusco-caeruleus Ota is guessed to be a melanoblast.

In consequence of making clear from what tissue this melanoblast developed, I have observed the pigment cells, as before-mentioned, which were in contact with nerve fibre and ran with the same bend to that of nerve. But the picture of such transition or affinity between this pigment cell and Schwannian cells as was seen in soft nevus was not found at all and the absolute inseparable state was presented. And this figure is accordant with that Itô[14] pointed out about the relation between pigment cell and nerve bundle in Mongolian spot and blue nevus, and consequently these cells are perhaps the cell connecting with the mesenchymal sheath of nerve (endo-perineurium). That the lesion of this nevus situated in the distribution of the 1st or 2nd branch of trigeminal nerve suggests the more the close relation with nervous system. The view that the pigment cell of this nevus is mesodermal is supported by the existence of complicated melanosis bulbi, which distributed in mesodermal tissue such as sclera, iris and choroid. Thereby I guess the pigment cell of this nevus also corresponds to mesodermal endo-or perineurium as Itô inferred about Mongolian spot and blue nevus, and blongs phylogenetically to the socalled "perineurale Pigmenthülle" of Weidenreich. Therefore, it is admitted that the histological picture, chemical nature and origin of this nevus resemble closely to those of Mongolian spot, and from the point of persistent and localized occurrence, I guess it is a kind of malformation similar to blue nevus. The differences from blue nevus are that nevus Ota predisposes the particular region which corresponds to the trigeminal nerve and complicates clinically frecklelike brown spot, histologically the increase of pigment granules in epidermal basal layer. The surrounding of orbit is the predisposing area where other pigmentation develops frequently and there exists perhaps many phylogenetic architechtonical factors such as shown in the fissurale theory of Virchow and it is supposed that this nevus belongs to the same principle. I have not enough data of explaining the pathogenesis of brown spot in this nevus but the positive Meirowsky's phenomenon suggests that epidermis is also the favorable part of oxidation.

Summary

110 cases of Nevus fusco-caerules ophthalmo-maxillaris were observed during recent eleven years in our department of dermatology, and its frequency is 0.4% to the whole outpatients. The site of nevus is characteristic and it is found in the region of the 1st or 2nd branch of trigeminal nerve and in the cases of about 61.3% melanosis bulbi was recognized. The alterantion of the color tones in deepness according to menstruation, seasons, surroundings or others was recognized in 18 cases. The angioneurotic disturbance in the lesion was recognized in the histamine scratch test.

In the histological and histochemical investigation the pigment cell resembles closely to that of Mongolian spot or blue nevus as follows, the hard fading with

hydrogen-peroxide and the positive oxydase-, peroxydase- and especially dopa-reaction suggest that the pigment cell is the mesodermal melanoblast, and it corresponds phylogenetically to the mesenchymal endo- or perineurium from the observation that the pigment cell has the close relation with nervefiber. Consequently, I think the etiological mechanism of this nevus is explained in our view of pigment formation that the quantitative alternation of melanin is due to the chemical transmission controlled by the vegetative nerve.

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VIII. Nevus Spilus en Nappe*

In a general way systematized nevus shows the unilateral and almost linear arrangement, and at times it involves more widespread area and especially we find rarely the lesion which consists of disseminated ephelidelike spots over the dark skin. In 1888, Jadassohn reported the "halbseitig localisiertes, ephelidenähnliches multiples Fleckmal" and in 1895 Jadassohn and Werner[1] discussed about the correlation between this pigmentation and the distribution of cutaneous nerve in 6 cases. Gougerot, Meirowsky,[2] Scholtz,[3] Crocker, Ehrmann, Duhring, Sprinz, Simon, Weidemann, Robinson and others also described the analogous cases. A female with the diagnosis of lentiginose profuse systematisée héréditaire by Aguilera and Cifrian[4] seems to be this disease. In Japan 3 cases of Matsumoto[5], Ninomiya,[6] one case each of Tanioku[7] and K. Kitamura[8] are reported.

Symptoms: According to the descriptions of the predecessors and our observations (see below), this affection is found frequenty in a slight dark complexioned persons rather than a blue-eyed White. The whole lesion is light brown or grayish black in color as Recklinghausen's freckles or pigmented spots. The border is indistinct or zig-zag. There are oval or polygonal irregular ephelidelike spots of millet seed to half rice grainsized over the lesion. The color of these spots varies from light brown like ephelides to blackish brown like pigmented spots and they do not elevate over the cutaneous surface. The density and arrangment of these spots are not constant and in exaggeration they give one the impression of the sesames which are scattered over a dirty table cloth. Furthermore some cases which show the slight hypertrichosis over the blackish brown skin seem to be a type of this affection.

Site of lesion: In general it takes place unilateral and spreads broad zoniform or napkin like in shapes over the neck, nape, shoulder, chest, back, upper arm (rarely on the forearm) and abdomen, and thus it seems suitable to call this as naevi spili zoniformes or naevi en nappes. The correlation between this nevus and the distribution of cutaneous nerve is as follows; the area of from C_3 to T_4—T_7 is involved most frequently, but in some cases the area of from T_{10}—T_{12} is affected. Therefore, there is close connection between this nevus and the cutaneous nerve. Thus this spot should be treated and classified as a kind of systematized nevi which are called nerve nevi.

Report of cases: Table VIII showed the patients of this nevus in recent 10 years in our clinic. No sexual difference was found. In most cases the disorder was found after birth but the nevi with slight color tone were noticed in puberty. Left side was oftener than right side and the cases with the pigmentation over the shoulder region were found most frequently. Two cases in Table VIII (16th and 17th case) were observed by Miura at the dermatological clinic of Nihon University and he offered us the specimens for biopsy.

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TABLE VIII

Case number	Age in years	Sex	Age of onset	Location	Comment
1	24	F		Left loin	Concomitant Bourneville-Pringle's disease
2	19	F	Inborn	Left neck, chest and back	No color-changes in menstruation
3	45	M	Inborn		
4	21	M	Inborn	Left chest, back and upper arm	
5	32	F		Left shoulder, axilla and upper arm	Concomitant chloasma gravidarum
6	28	F		Left shoulder and upper arm	Antipyrine fixed eruption over the center of the nevus
7	48	F	Inborn	Left back	Quite same distribution to herpes zoster on the right side of the back
8	22	F	Inborn	Left neck, shoulder, chest and upper arm	
9	51	F	Inborn	Right chest, back, thigh and abdomen	
10	21	F	Inborn	Right neck	
11	26	M	Inborn	Right chest (on the niveau of both nipples)	
12	18	M	About 13 years	Left shoulder and back	
13	26	F		Right abdomen and flexor-surface of thigh	
14	20	M	Inborn	Left cheek and neck	His father has pigmented moles and his mother has ephelides
15	20	M	About 7 years	Left chest, shoulder and thigh	
16	21	M	About 18 years	Right shoulder and extensor-surface of upper arm	Hypertrichosis of the central part of the nevus
17	23	M	About 19 years	"	Hypertrichosis of the central part of the nevus; consanguineous marriage among parents of patient
18	17	F	Inborn	Left shoulder and extensor-surface of upper arm (see Plate X, Figure 41)	
19	22	M	About 17 years	Left shoulder, axilla and flexor-surface of upper arm	
20	22	M	Inborn	Radial surface of the back of left hand	Hypertrichosis of the central part of the nevus
21	31	M	Inborn	Left chest, right forearm	Several depigmentations on ileogluteal region

Clinico-biological Investigations: a) Sensory and Diaphoretic Tests—Disturbances of sensations were found in 5 cases out of 21 cases and diaphoretic disturbances were found in 2 cases out of 4 tested cases (Table IX).

TABLE IX

Case number	Local disturbances of sensation	Local disturbances of diaphoresis
16	Hypesthesia, hypalgesia and thermohypesthesia	No difference of diaphoresis between normal skin and the nevus
17	Hypesthesia, hypalgesia and reduced feeling of cold but normal of heat	
18	Thermohypesthesia, normal sensation of touch and pain	Pilocarpine method showed slight sooner diaphoresis in nevus rather than in normal skin
19	Thermohypesthesia in the central part of the nevus	No diaphoresis in 14 minutes after pilocarpine method in the central part of the nevus
20	Hypalgesia in the central part of the nevus and normal feeling of temperature	Adrenalin method (Wada) showed no distinct difference of diaphoresis between normal skin and the nevus

b) Histamine Scratch Test

TABLE X

Case number	The differences in the development of wheal between normal skin and nevus
5	Simultaneously (100 seconds)
8	Simultaneously (80 seconds) then erythema diffused over the nevus
9	Simultaneously (80 seconds)
15	Simultaneously (165 seconds) but wheal over the nevus was faint
18	Simultaneously (90 seconds)
19	Wheal developed over the nevus later and disappeared sooner than the normal skin
20	Wheal developed at the 9th minute over the nevus and existed for 12½ minutes
21	Wheal developed at the 130th second over the nevus and existed for 30 minutes

The fact that in the 6th case a coin-sized antipyrine fixed eruption was found on regio acromialis which corresponds to the center of the nevus was also the suggestion of the disturbance in angioneurotic nerve.

Heredity: Siemens[9] described the frequent family occurrence of nevi pigmentosi spili, he noticed the concordance in 21 cases out of 56 twins. We noticed the pigmented spot and ephelides in parents of patient in the 14th case and the consanguineous marriage among parents of patient in 17th case but could not observe the certain evidence of inheritance at all.

Histological studies: Four cases (16th, 17th, 19th and 20th case) were studied. The principal alteration was the increase of melanin granules in basal layer, esp.

abundant at the top of epidermal ridges and sweat-pores. Furthermore they diffused in two or three layers of prickle cell layer. Epidermal ridges became slender, basal cells were prismatic and arranged like a palisade. At times the perinuclear vacuolization of Malpighian rete cells was found. No alterations were found in granular and horny layer. Papillae were projected distinctly equivalent to the epidermal ridges, and dilated blood capillaries were filled with blood cells. The blood vessels of subpapillary and rete layer dilated also and the slight perivascular round-cell infiltration was noticed. Chromatophore was relatively increased in number in papillary and rete layer. But nevus cell could not be found anywhere. The sweat-duct and sweat-gland slightly dilated but sebaceous gland was normal. The significant changes in connective tissue or elastic fiber could not be found approximately.

Diagnosis: Jadassohn concluded that these nevi are the frecklelike and Ehrmann considered them chloasma-like pigmentation. In the schema which was established by Pollio[10] these nevi are classified into the pigmented nevus-like Recklinghausen's disease. But this disease has a somewhat indistinct bordering and unilateral arrangement. Furthermore, histologically, these nevi have no nevus cells and they are easily distinguished from soft nevi. Thus we consider them as a type of systematized nevi and the signature nevus spilus en nappe may be the most suitable one for their clinical manifestations.

Comment

Concerning the pathogenesis of systematized nevi the participation of nervous system has been studied by many investigators, although we have not come to a definite conclusion as yet. Since the neurogen concept of Bärensprung[11] (1863) Grosser, van Rynberk, Haecker and others studied the pigmented flecks of animals. In French school Philipson,[12] Hallopeau,[13] Gougerot and Noce, Klippel and Weil[14] mentioned the neurogen theories and metameric theory of Alexander and Blaschko,[15] phylogenetical observation of Meirowsky or Jadassohn were reported. These are suggestive of concerning of cutaneous nerve with the pathogenesis of nevus, but several investigators maintain that the nevus does not always coincides with the distribution of cutaneous nerve in arrangement. But it seems difficult to arrive at a single conclusion because of reasons such as complexed peripheral innervation of skin, interference of vegetative and sensible nerves, individual variations and the difficulties in the histological and physiological investigation of cutaneous nerves. Therefore following cases are mentioned as the clinical and complemental evidence of this subject; nevus-like pigmentation caused by bullet (Falkenstein),[16] linear nevus after breast injury (Nontkowsky), pigmented nevus after neuritis (Meirowsky), linear nevus accompany with the anomaly of cervical-rib of same side (Lévi and Tzanck[17]), systematized nevus accompany with sclerodermoid and pain (Queyrat, Lévi and Rabut[18]), nevuslike pigmentation with circumscribed disturbance of sympathetic nerve (Laignel-Lavastine), etc. and especially a female observed by Lewith[19] is interest. The patient presented those homolateral sensible and trophoneurotic disturbances such as thermohypesthesia which coincident to the flat pigmented nevus over right half of chest and upper extremity (C_4—D_7) and increased sweating of right axilla, asymmety of face, anisocoria, hypoplasy of right mamma,

hypertrophy of left deltoideus and left handedness. And Lewith pointed out the conception of Kyrle which mentioned that the sympathicus regulates the pigment formation and emphasized the close correlation between pigmented nevus and homolateral nervous disturbance. Recently Horiuchi[20] reported a case of pigmented nevus accompanied with localized hyperidrosis and we remember the several cases reported by Eppinger which have unilateral pigmented nevus and malformed kidney of the same side.

We noticed in this article the homolateral nervous disturbances (reduced sensibilities, sweating anomaly and the notable differences of histamine wheal between nevus and normal skin) in the abovementioned nevi spili en nappes and especially in our 7th patient the nevus showed quite same arrangement and distribution but in opposite side to that of herpes zoster. This observation corresponds to that of Bärensprung and Simon[21] in which systematized nevus arranged coincidently to the distribution of herpes zoster and they surmised the alteration in ganglion.

Moreover in the histological investigation of pigmented nevus, Mongolian spot, blue nevus and nevus fusco-caeruleus we have obtained the data which indicate the relation to the neurofibril, thus we think much of the participation of nervous factor in the pathogenesis of systematized pigmented nevus.

Summary

The cases of nevus spilus en nappe which is a type of systematized nevus were reported and the homolateral nervous disturbances were noticed. Thus we believe that these observations are the best materials to suspect the concept of participation of nervous element in the pigment formation.

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IX. Nevus Depigmentosus en Nappe*

In the Negro, Meirowsky[1] reported the cases which resemble the nevus systematicus depigmentosus but such cases are rare in Japan. Moreover vitiligo presents occasionally systematized arrangement and thus the diagnosis is to be established after careful examinations.

This study is based on the observations of several cases of nevus systematicus depigmentosus which are arranged similar to the nevus spilus which was studied in the preceding report.

Case 1.—Male, aged 27, seen at our clinic on Oct., 1950 suffering from the depigmentation over the shoulder region which becomes apparent a half year after birth. His maternal grandfather had pigmented moles. Examinations: The moderate stature and well nourished. A pale depigmented lesion (C_{IV}—C_{VI}) over the right scapular region to the regio deltoidea and faint brown frecklelike spots in size of millet-seed were scattered over this area (Plate X, Figure 42). The bordering of the area was zig-zag and the depigmentation was light in color in the area from the median margine of right scapula to the median line and thus the bordering to the normal skin was not so distinct. Furthermore several blackish brown lentigolike spots were indicated over this area. No erythema or squama was noticed and no paresthesia or hypesthesia was observed. The histamine scratch test showed no differences between the normal and the depigmented skin. The pilocarpine test (Minor-Wada's method) showed the fast diaphoresis by 30–60 seconds in the depigmented area but the difference in degree of diaphoresis was not found. Aschner's phenomenon negative, basal metabolic rate +5%. Increased urobilinogen in urine.

Diagnosis: The disorder is inborn and distinguished from vitiligo because of the absence of hyperpigmented areola. In lepra the presence of disturbances in sensations and thickend nerve readily establishes the diagnosis. From Recklinghausen's disease this can be distinguished by the zig-zag bordering as the significant feature of systematized nevi which was mentioned by Siemens and there was of course no neurofibroma. Thus we think the nevus systematicus depigmentosus en nappe should be brought into the diagnosis of this depigmentation.

Case 2.—Female, aged 26, consulted our clinic at first on July, 1950. Examinations: Physical examination negative. She presented the nevus fusco-caeruleus ophthalmo-maxillaris Ota over the right temple and cheek and the irregular palmsized depigmented lesions over the radial side on the flexor surface of right forearm. The pigmented spots brown in color, varied in size from that of half rice-grain to a lentil were scattered over this area. Moreover several irregular coinsized depigmentations were jointed with each other over the flexor surface of the right upper arm to the distribution of n. cutaneous brachii medialis. Further each one depigmented spot of palm in size were found over the right mamillar region (T_{III}—T_{IV}) and the flank

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(TIX—TX). These depigmentations were light and not so distinct in color as much as vitiligo and no erythema or squama was found over these spots. The histamine scratch test was performed at the most distinct spot over the forearm and the wheal developed after 1½ minutes on both normal and depigmented skin. The local injection of hypophysis hormone and hematoporphyrin did not develop the pigment.

Case 3.—Female, aged 20 seen at first on Aug., 1947. She stated that since 3 years she has had the several dotted leucodermas over the left thigh and that they increased in number recently. Examinations: Blood count normal, urinalysis negative, regular menstruation, basal metabolic rate +14%, Aschner's phenomenon negative. Ephelides on the face. Many dotted depigmentations varied in size from that of a millet seed to a half rice-grain, follicular or not, arranged in groups, were found over the area from median side of knee downwards obliquely to the front surface of left leg. The bordering was distinct but no hyperpigmented areola, erythema or squama was seen over the depigmentation. The entire lesion corresponded to the distribution of rami cutanei cruris medialis (LIV). The histamine scratch test showed the wheal development at 1½ minutes over the normal skin but no wheal was noticed after 3 minutes in the depigmented area. Histologically, the depigmented skin showed decreased melanin granules in basal layer of epidermis than normal skin and in some part they were disappeared entirely. Moreover in this part the basal cells were shortened and swollen and their nuclei were ovoid in shape and the perinuclear vacuolization, furthermore mélanoblaste épuisé (Masson) was noticed. Thus these basal cells were distinguished from the normal palisadelike arranged cylindrical basal cells because of their disarrangement. This finding was most evidently observed in the silver impregnation, that is to say, the basal cell layer seemed like a clear unstainable zone. The blood capillaries under the depigmented area dilated moderately and the perivascular zone seemed to be spongious but there was no cell infiltration. The other parts in epidermis and corium were normal. The elastic fiber and Gitterfaser showed no alterations. The local injection of hematoporphyrin and Vitamin B_2 in the depigmented area was not efficient.

Case 4.—Female, aged 16, was first seen in our clinic on Aug., 1941. The patient noticed the verrucous eruptions over the neck since 1 year of age. Examinations: Physical examination negative. Urinalysis negative. A nevus systematicus acneiformis over the left foreneck in the oblique direction. Furthermore a depigmented spot in size of a coin was situated under lateral of it. The entire lesion corresponded to the distribution of CIII. The histamine scratch test over the nevus acneiformis indicated the somewhat dilayed development of wheal and no wheal was found over the depigmented area.

SUMMARY

4 cases of nevus systematicus depigmentosus were reported. In these cases there were no atrophy which was mentioned by Leven[2] in nevus linearis atrophicus depigmentosus and thus we think that they were surmised as a simple depigmented nevus. But the facts that the nevus takes place over the definite distribution of cutaneous nerve and that they indicated some significant differences against normal skin in the histamine scratch test are suggestive of the homolateral disturbances of nervous system. We consider them as the favorable data for our theory of melanin

production.

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X. Incontinentia Pigmenti (Bloch-Sulzberger)*

In 1925 Bloch[1] reported a young female case of "hitherto undescribed congenital pigmentary disturbance" under the title of "incontinentia pigmenti." Sulzberger[2] made in Bloch's clinic an extensive report on this subject in 1928. Subsequently, Naegeli[3] demonstrated an analogous case with family occurrence and named this chromatophore nevus. Siemens[4] in 1929 presented a case under the name of melanosis corii degenerativa. His case was admitted by Sulzberger as belonging to this disease. Later Bardach[5] noticed similar cases in twins. The identity of his cases with former cases was eagerly discussed by Siemens and Meirowsky-Leven and thus attracted the attention of all investigators in the world. The number of this rare condition reported in the literature amounted to about 40. The first case in Japan was observed by K. Kitamura[6] and at the 250th meeting of the Tokyo Dermatologic Society Yokoyama[7] demonstrated the second case, and the present author observed 3 cases. These are illustrated in Table XI.

TABLE XI

Patient No.	1	2 (Plate IX, Fig. 34)	3 (Plate IX, Figs. 35–37)
Sex	F	F	F
Age	11 months	7 months	26 years
Consanguinity and family occurrence	None	None	None
Onset	10 days after birth	Existed by birth	100 days after birth
Initial eruption	Erythematous eruption with intermittent fever	Erythematous exanthema	Varicelliform eruption
Symptom	Mortar splash-like pigmentation dark brown in color with undulated zonal arrangement. Zebra-like appearance	Bluish-brown splash-like pigment patches with marble configuration	Slaty ashy brown pigmentation stellate in form
Location	Symmetrically generalized	Symmetrically generalized	Systematized on the breast, shoulder and flexor surfaces of extremities
Other symptoms	Stupid appearance. Slight microcephalia and sudden death by pneumonia	Congenital nucleal cataracta of the left eye	Asymmetry of the mamma

Clinical Appearance: In typical cases stellate mortar-splash-like pigment patches varying from slaty ashy brown to bluish lead-hued in color, are observed in reticulated arrangement. In cases reported by Siemens, Kitamura, Schuermann and Yokoyama, systematized arrangment is ascertainable. In my first case I de-

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scribed exaggeratedly as zebra-like appearance. So it is natural that the differentiation from systematized nevus was eagerly discussed in Bardach's case. The surface of the lesion is usually flat but sometimes is somewhat elevated or slightly atrophic and dent. Occasionally desquamation may be present. At lower abdomen and ends of extremities the lesion may take verrucous appearance. In most of the cases the eruption is more or less generalized, but the severity may differ on the left and right side.

Inflammatory Changes at the Onset: It was already noticed since Bloch and Sulzberger that such inflammatory changes as erythema, swelling, lichenoid eruption or zosteriform vesiculations may be accompanied at the onset. This is observed in almost all cases except for the cases of Naegeli, Moncorps, Nexmand. However, no investigators attached great importance to this fact, till Heilesen[8] emphasized its significance. Uebel[9] *et al.* paid attention to the fact that in his case the mother had suffered from febril disease in the 6th month of pregnancy. He surmised that there exists some causal relationship just as the incidence of malformations through infection or intoxication of the mother in the fetal period. Thus he emphasized the rôle of maternal influence during pregnancy and called this embryopathia via adrenalsystem. Similar case was reported also by Haber.

Clinical Course: The clinical course is somewhat characteristic, *i.e.* the primary onset is noticed soon after birth and the lesion which becomes gradually intense till in 4 or 5 years of age begins to subside untill complete recovery. This peculiarity in the clinical course as well as the inflammation at the onset is regarded as important differentiating point from systematized nevi. Sulzberger's[10] case and my third case in which the eruption persisted till 19 and 26 years of age respectively are exceptional ones in this point.

Complications: From the report of Bloch-Sulzberger the complications of various congenital malformations espec. abnormalities in the psychic, nervous and optical systems were described by many authors. Most often debility, nervous irritability, microcephaly, retrobulbar glioma, strabism, nystagmus, spasm of extremities and heart failure are reported. Especially in Sulzberger-Fraser-Hutner's case congenital anopsy and ectodermal dysplasy are associated, and in Heilesen's hare-lip and convergent strabism are noticed in the family. On account of this frequent combination with various malformations in ocular system, Uebel, Ludwig and Korting proposed a hypothesis concerning to its etiology in reference to dysgenesis mesodermalis corneae et iridis of Rieger or retrolental fibroplasia of Terry. In my second case congenital nuclear cataracta was associated.

Heredity: In the case of Naegeli father and two of daughters were affected. Sulzberger's case originated from a family with female-linked congenital ectodermal dysplasia. Further Sobel[11] observed a case of a young Chinese girl who's father presented suspicious eruption for incontinentia pigmenti. In Japan Kitamura[12] reported two cases with family occurrence. Anyway the onset in infancy and the frequent combination with congenital anomalies suggest that incontinentia pigmenti has at least partial relationship with congenital factors. Nearly all of the cases reported in the literature are female cases except for Moncorps's case. This, too, is interesting in the genetical survey of this disease.

Histopathology: The characteristic histological change is the increase of pigment

cells in the upper dermal layer. They are stellate or fusiform and are located mostly near blood vessels and contain large amount of coarse melanin granules (Plate X, Figure 39). Their dopa reaction is negative and they are held to be endothelial or connective tissue cell by Bloch-Sulzberger, though recently Carol and Bour[13] obtained positive dopa reaction in these cells. In the basal layer in the pigmented area, the melanin content is rather decreased and existing melanin granules are smaller and various degenerative changes such as vacuolization in the protoplasm and pycnotic nuclei are noticed in the basal cells (Plate X, Figure 40).

The inflammatory changes in the pigmented area consist of cellular infiltration of connective tissue cells and lymphocytes, plasma cells and edema of papillary layer and dilation of blood vessels. And eosinophilic cells are observed by Kitamura, Carney[11] and Yokoyama. The former two described also intraepidermal vesiculation containing eosinophilic cells. Uebel *et al.* called attention to the swelling of endothel cells of arterioles in the cutis, and suggested some relationship with allergic-hyperergic inflammation like periarteritis nodosa. In my histological study of the third case no inflammatory changes are noticed as in Sulzberger's case. It is, however, quite natural that inflammation shoud have subsided after such a long interval.

Doornink[15] studied histologically on pigmentation in Riehl's melanosis, poikiloderma, lichen ruber and drug eruption and obtained similar results as in incontinentia pigmenti. Thus he came to the conclusion that the term incontinentia pigmenti is not the name of special disease but a histo-pathological symptom.

Comment

On the ground of the histological study and the dopa theory, Bloch and Sulzberger explained the pathogenesis of this disease in the following manner. In the normal epidermis the melanin which is produced in the basal layer is mostly transported to the upper layer. In this condition the epidermis has become incontinent of the pigment and the melanin granules drop down to the corium and are phagocyted by chromatophores. This theory was also supported by the experiment of Miescher who by injection of dopa melanin in the vitiliginous area observed that artificial melanin is sustained for a long time in the chromatophore. Siemens accepted Bloch's theory, but at the same time assumed that the melanin in basal layer is increased in view of the darker skin color. In my material I obtained analogous results to Bloth-Sulzberger's. The existence of argentaffin granules in the basal layer as well as the negative dopa reaction is in favor of his theory. Further the frequent observations of compensatory increased dermal pigmentation in cases of scanty pigment content in the epidermis in animal kingdom may support this view. However, owing to the rarity of this disease there remain many questions which are to be solved. Especially the significance of the inflammation at the onset is not thoroughly understood. It is true that inflammation may activate pigment production as in the cases of tar spread, arsenical intake or actinodermatitis. However, it is impossible to attribute such generalized occurrence of inflammation vaguely to congenital factors only. In the following the author tries to investigate this problem chiefly in connection with the study of the nervous system.

1. The peculiar systematized arrangement of this disease was already admitted in the discussions of Bardach's case. The participation of cutaneous nerve is natural-

ly suspected as in systematized nevi. So the Voigt's border line of the termination of nerve or Blaschko's dermatom arrangement line is proposed to be the site of predilection. The location in typical cases is in arciform from the breast region to the shoulder and descends along the upper arm, quite corresponding to the Voigt's line.

2. In usual dermatoses the eruption is convex-bordered in accordance with a vasomotor nerve, while in this disease the morphology of the larger eruption is characteristically concave contoured. How is this concave form explained? Here I tried to find some special significance. First of all the reagibility of the vasomotor nerve was examined by histamine scratch test. In the pigmented area the development of the wheal was earlier than the surrounding normal zone by 10–15 seconds. This fact suggests that there exists congenital abnormality either morphological or functional in the distribution of the vasomotor nerve especially in the constrictor portion. This interstice in the distribution of the vasomotor nerve is the site of predilection and thus the typical concave form is explained.

3. From above mentioned hypothesis the occurrence of inflammatory changes at the onset is explained in the following manner. In individuals possessed of congenital labil autonomic nervous system there may arise abnormal reactions to external stimuli of the extrauterine environment. These reactions give rise to inflammatory changes especially in the interstice of the vasoconstrictor portion and thus result in the disturbance of pigment metabolism. The abovementioned acceleration of the histamine test may have disclosed the remainder of this local disposition. And, further, eosinophilia noticed by Kitamura may be interpreted as a manifestation of abnormality of tonus of autonomic nervous system. The maternal influence in fetal period and the histological resemblance with allergic inflammation of the arterioles mentioned by Uebel and others wait for further confirmation and will not be touched here.

4. The pigmentation in this dermatose usually disappears by 4–5 years of age like Mongolian spot. This is explained either as the common characteristics of dermal pigment cells or as the results of compensation through the regulatory function of peripheral autonomic nervous system in the loco or through chemical transmission. This is quite corresponding to the observation of Lassar and others that systematized nevi may disappear by itself . . . the characteristics of socalled nerve nevus.

In my third case as well as in Sulzberger-Fraser-Hutner's the pigmentation persisted till the youth like the persistence of Mongolian spot in form of blue nevus.

5. In all reported cases the pigmentation is most prominent in the perimammilary region, and there the eruption persists longer than in other area. In the case of Sulzberger-Fraser-Hutner the breast of the diseased side was smaller than the other. In my third case the left mamma where the pigmentation was much darker, was by far smaller than the right (Plate IX, Figure 36). I assume that this indicates the retarded development of this merocrine glands in the sense of homolateral nervous disturbance, *i.e.* congenital asymmetrical distribution of trophic nerve. In accordance with this theory I observed two female cases of systematized nevi in the breast region with smaller mamma of diseased side. In another case of a 20 years old girl with hairy nevus on the breast asymmetry of the mamma was noticed. These facts as well as Lewith's[11] similar observation are in favor of my foregoing assumption.

The relationship between melanin production and nervous factor has been established by the experiments of Brücke, Pouchet and v. Frisch on the chameleon and turbot. On the ground of these investigations Parker in 1932 proposed the theory of neurohumoralism. In human being many observations suggest that there exists correlation between nervous system and melanin production. The recent observation of Miura[17] of the pigment borderline on Bolk-Sherrington-Foerster's axillary line is the full suggestion of this point. The present author has already reported many observations regarding this problem.

Summary

This disease occurs in individuals with congenital dysfunction on the part of autonomic nervous system. These easily react with various grades of inflammatory changes against external stimuli of the extrauterine environment. The inflammation results in the disturbance of melanin metabolism and further in the incontinence of the epidermis. Thus the pigmentation is produced in the characteristic symmetrical and concave arrangement. But the recovery can appear sooner or later when the interstice is compensated by the interaction of peripheral nervous system.

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XI. Incontinentia Pigmenti Achromians

A Singular Case of Nevus Depigmentosus Systematicus Bilateralis*

Case

A 22 years old Japanese girl. Date of the first consultation Oct., 1950. Her father died of stomach ulcer. No dermatological disorders in the members of the family. She had had often exanthemas on various parts of the body and suffered from chilblain for the past few years. Already in the childhood a leucodermatous patch appeared on the breast which grew more marking gradually and since two years extended to the upper arms on both sides.

Clinical and laboratory examinations: Well built and good nourished. Menstruation regular. Blood picture no deviation from normal. Urin and feces normal. B.M.R.+17%. Aschner's phenomenon positive. Mantoux reaction positive. Wassermann reaction negative. Ophthalmologically no pathological finding.

Cutaneous changes: The entire skin surface was somewhat dry, and slight follicular hyperkeratosis was noticed on the elbow, shoulder and nates. On the trunk and extremities symmetrical leucoderma with systematized arrangement was noticed. On closer observation, most prominent was the one on the upper half of the body. Beginning from the breast and till the flexor surface of the upper arm, the skin looked as if the normal pigment was brushed off. Upper and outside of the breast region on both sides, there were many irregular shaped, zigzag bordered mortar splash-like depigmented spots (Plate IX, Figure 38). Several lines of islets of leucoderma ran laterally beginning from these spots. They ran along the axilla and medial of upper arms down to the middle of forearms on the ulnar side where they vanished gradually. Other lines of islets were arranged on both sides of the breast to the back sporadically. Similar zoster-like arrangement was observed more clearly on the abdomen and, to some extent, at inguinal, gluteal and femoral regions. In short, all patches of depigmentation were arranged in accordance with Voigt's demarcation line of cutaneous nerves.

Depigmentation was most remarkable on the breast and upper arms, where it could be easily distinguished in this case who had ordinary complexion of our race. But on the lower half of the body, the depigmentation was rather obscure and might be overlooked at the first glance. The morphology of each spot was concave bordered without halo of hyperpigmentation in contrast to vitiligo. Inflammatory changes such as erythema and desquamation were completely lacking. On the breast where the depigmentation was most prominent, some of the spots showed slight depression in appearance resembling atrophy. In general, leucodermatous area was somewhat wider on the left side of the body than on the right, and, in addition, the mamma of the left side was smaller than the right.

Biological experiments: 1. Histamine scratch test was made at the leucodermatous

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* By Minor Itô.

area including surrounding normal skin with 0.1% solution of histamine hydrochloride. Erythema was noticed after 20 seconds. After 2 minutes and 15 seconds, wheal formation in the former part by 15 seconds later than in normal area, but after 3 minutes and 40 seconds both wheal showed same intensity. The disappearance of the wheal occurred in 50 minutes at the diseased site and in 90 minutes in the normal. Dermographism was negative at each site. 2. Diaphoresis was examined by Wada's modification of Minor's method. Diluted tincture of iodine was applied to the skin and after drying emulsion of starch in castor oil was spread. Then 0,6 cc. of pilocarpine hydrochloride was injected hypodermally. In leucodermatous patches no change was noticed after 30 minutes, while in the surrounding area innumerable black dots appeared in 10 minutes. 3. Capillary resistance was examined by the suction method similar to that of Hecht. The control datum was 13 cm Hg. at infraclavicular region. By 18 cm Hg. 10 bleeding dots were observed in the depigmented area in contrast to 1 in the neighbouring zone.

Histopathology: Histological specimen was excised from the left upper arm. In the epidermis no changes were noticed in the horny, granular layer and in the rete. In the basal layer, melanin granules, which were evidently identified in the normal part, were completely lacking. Even with silver impregnation method no black granules were noticed in the leucodermatous area. And there some basal cells were slightly disordered, with their swollen nuclei and protoplasm and with perinuclear vacuole formation. However, the total thickness of the epidermis and the form of epidermal processes were normal. Chromatophores in the surrounding zone was located mostly perivascular and apart from these free melanin granules were scattered here and there. In the diseased part neither chromatophores nor free granules of melanin were found, and the capillaries in the papillary layer were slightly dilated with their swollen endothels and connective tissue fibers apparently scarce. By soaking the slice in hydrogen peroxide solution for 24 hours the melanin in the basal layer faded out completely while that in chromatophores turned to pale yellow. No morpholigical changes were seen in nerve fibers with Masson's staining.

Diagnosis: It is easy to differentiate this from leucopathia reticularis et punctata symmetrica Matsumoto[1] and from vitiligo on account of the clinical picture. It is proper to enlist this in the group of nevus, nevus depigmentosus systematicus bilateralis. In this case, however, the arrangment of depigmented spots conforms in toto with that of the third case in my former report on incontinentia pigmenti[2] —only in this case the pigmentation was displaced by depigmentation, the negative picture of incontinentia pigmenti. So it is easy to remind one of the clinical picture of this case by naming this incontinentia pigmenti achromians, though there is some curiosity of this denomination.

Comment

In my former report I tried to explain the pathogenesis of incontinentia pigmenti in the following manner. In individuals with unstable vegetative nervous system, there may arise abnormal inflammatory reactions to external stimuli of the extrauterine environment through the dysharmony of vasomotor nerve. These are followed by the disturbance of melanin metabolism and consequently gives rise to concave

contoured pigmentation on the Voigt's line. But sooner or later spontaneous recovery is procurable by the compensation through the peripheral nervous system.

The case presented here is quite persuasive of this assumption. The dysfunction of vasomotor nerve is disclosed by the results of histamine scratch test, retarded perspiration and by reduced capillary resistance. And the concave contour and the unique arrangement suggest us that Voigt's line is the site of predilection and further that this disease had occurred on the congenital abnormality in the distribution of the termination of vegetative nervous system in Voigt's borderline. My observation, in common with the results of animal experiments of Brücke, Pouchet and v. Frisch (hyperpigmentation after sympathectomy) and the idea of pigment borderline of Miura,[3] is in favor of my advocation—the participation of vegetative nervous system in pigment production.

The asymmetry of the breast glands in this case as well as in Sulzberger-Fraser-Hutner's[4] and my third case of incontinentia pigmenti and the three cases of pigmented hairy nevus in my previous report, is assumed to be indicative of the retarded development of the merocrine gland through the congenital asymmetry of trophic nerves in the sense of homolateral nervous disturbance of Kyrle or Lewith.[5]

Summary

A case of systematized depigmented nevus is reported, which shows, as it were, the negative picture of incontinentia pigmenti. Such incidence is in favor of my theory, the participation of the vegetative nervous system in pigment production.

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XII. Malignant Melanoma*

Research on the melanoma was initiated by Laennec (1806). Müller considered it to be a kind of carcinoma. But Virchow pointed out the existence of melanosarcoma and asserted that there was the mixed form of melanosarcoma and melanocarcinoma, namely melanocarcinosarcoma. Since then, this problem has been discussed by many authors. Ribbert asserted that melanoma was chromatophoroma and Lubarsch declared that it was melanoblastoma. Miescher described that it was proper to adopt clinically the term melanomalignoma. Thus the pathogenesis of melanoma had been discussed in association with the problem of the genesis of nevus cell in pigmented nevus, Mongolian spot cell and blue nevus cell, but there are still many differences of opinion. In U.S.A., too, many authors discussed this problem in "The Biology of Melanomas" published from the New York Academy of Sciences (1948).

According to Miescher's description, the frequency of socalled melanomalignoma, was 35 cases in 686 cases of cancer of the skin and the disease showed the initial symptom in the middle age, rarely in childhood (Darier and Civatte[1]) and in a suckling who was born from the mother of melanomalignoma (Weber, Schwarz and Hellenschmied[2]). In Japan, Kawanishi[3] (1928) collected 44 cases and Ozaki[4] (1933) 28 cases of melanomalignoma. Watanabe[5] in our clinic collected 23 cases in Japanese literature from 1924 to 1938 of which three cases were reported histologically as cancer and others as sarcoma.

The locations of this disease can be distributed in the skin of whole body. On the head and face, they originate mostly from the lentigo. On the lower extremity especially on the foot, they present frequently ulcerated type and the subungual cases call attention as a specific variety. Recently Pack[6] compared the frequency of the distribution of pigmented nevi with that of melanomas. According to his description, the regional distribution of pigmented nevi and melanomas over the skin is not exactly the same. Both tumors occur with considerable frequency on the face and neck, and equal frequency over the skin of the trunk. Pigmented nevi are not commonly found on the feet and on the genitals, but the frequency of melanomas in these specific locations is relatively high.

Their clinical and microscopical findings are different from each other corresponding to their locations. The prognosis is very various too. Some of them are very malignant: their metastasis is very fast and then there occur generalized melanomatosis and melanuria.

We want to report our recent cases with the object of histological and histochemical studies of melanin production.

Melanocarcinoma on the Face

The reports of the melanocarcinoma on the face are found frequently. In

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Japanese literature, too, there are many reports of melanoma originated from the lentigo or from the pigmented nevus on the face. Their prognosis is not so unfavorable. Most of them can be easily diagnosed as carcinoma by the histological examinations. As we think it unnecessary to discuss their pathogenesis especially here, we show five cases which were experienced by Itô, one of the authors, in his clinic of Kanazawa Medical College, Japan (Table XII).

TABLE XII
Melanocarcinoma on the Face

Age	Sex	Originated from	Accelerated by	Onset age	Symptoms	Localization	Histological findings	Treatment
61	M	Pigmented nevus	Rubbing	50	Black, papillary	Right external canthus	Basal-cell carcinoma	Extirpation cured
65	F	Lentigo	Scratching	62	Ulcerated, lentil-sized	Left nasolabial fold		
65	F	Lentigo		56	Black, ulcerated, compact	Right medial canthus	Cancroid	Extirpation
54	M	Lentigo		52	Black, papillary	Right upper eyelid	Cancroid	
38	F	Pigmented nevus	Medical cauterizing	31	Black, papillary, coin-sized	Right cheek	Basal-cell carcinoma	Extirpation cured

Generalized Melanocarcinoma

The metastasis of the malignant melanoma is very rapid and it shows often the generalized melanoma, in which the skin and many internal organs are invaded. According to Watanabe in our clinic, 7 cases out of 13 cases, in which the descriptions of the metastasis were clear, showed the generalized metastasis. Nevertheless, the detailed report of these cases is relatively rare. Way and Light[7] reported a case, in which cutaneous metastasis was unusually extensive. Oka[8] described a similar case and discussed especially the melanuria. The most interesting in these cases is the presence of the melanotic and amelanotic tumors. We experienced a similar case recently.

Case Report: A veterinarion, aged 65. First examination in 1951. He had remarked a smooth, pinhead-sized, blue spot on the anterior surface of the left thigh since childhood, which had produced no subjective symptom. About half a year before, he caught cold and injected "Multin" himself on the place of previously described bluish pigmented spot. Next morning he noticed the reddening on the injected place. The reddened place around the pigmented spot so changed gradually into vitiligoid macula that there are presented a Sutton's leucoderma-like figure as a whole. At the same time, he noticed the leucoderma on the both sides of the preauricular regions, which enlarged gradually and reached about 3 cm in diameter. After several days from that time, he noticed numerous subcutaneous tumors, varying from 0.3 cm to 1 cm

in diameter, on the left inguinal region and the anterior surface of the left thigh. These tumors enlarged in size and increased in number, spreading over almost whole body. On January 28, 1951, he had five fingertip-sized tumors extirpated on the left inguinal region and the part of Sutton's leucoderma in the surgical department of our hospital. Since that time, the tumors on the whole body enlarged in size and increased in number more and more. On April 14, 1951, he came to our clinic. Status presens: A poorly nourished, anemic old man. Blood sedimentation rate (70–95), blood Wassermann's reaction negative. There are the leucodermas on the both sides of preauricular regions and a pinhead-sized lentigo on the brow. Numerous subcustaneous tumors, varying from red-been to pigeonegg in size, are visible and palpable on the whole body, esp. on the left inguinal region. The majority of them does not adhere both to upper skin and to their bases. Some of them present bluish tone. Several fingertip-sized, hard lymph nodes on the right axillar, both sides of inguinal and left femoral region are palpable. Course: These tumors on the whole body especially on the left inguinal region enlarged in size and increased in number gradually. Some of them adhere to their upper skin where slight reddening are found. He complains severe, to the left leg running pain. The anemia and urobilinogen in the urine became more remarkable. But blood Takata-reaction and melanuria remained negative throughout the progress. X-ray examinations showed small quantity of effusion of the both sides of pleura and slight stenosis of the pylorus. On gastric juice examination, hypoacidity was presented. The occult blood tests of the gastric juice and feces were positive. Electrocardiogram in about 24 hours before his death, showed the figures of myocardinjury. He died in May 26, 1951.

The autopsy was performed and showed following findings: numerous tumors in the subcutis and muscles of whole body and multiple metastasis on the liver (amelanotic), spleen, mucous membrane of stomach, duodenum, jejunum, ileum and rectum, serous membrane of bladder, kidneys (but a few and amelanotic), thyroid gland, right lung, peri- and myocardium and lymph nodes of left pulmonary hilus, mesenterium, retroperitoneum, neck, left subclavicular, axillar and inguinal regions.

Histological and histochemical findings: 1) Sutton's leucoderma on the anterior surface of the left thigh. On the leucodermal place, there are no change in the epidermis, no melanin granules in the basal cells and slight inflamatory changes but no chromatophore in the corium. The bluish pigmented spot, situating at the center of the leucoderma, presents no change in the epidermis and no pigment in the basal cells too. But there are nevus cell nests which are circumscribed in the reticular layer of the corium with slight round cell infiltration in their circumferences. These nevus cells contain relatively smaller pigment granules. These findings just correspond with that of Sutton's leucoderma.

2) Amelanotic tumor on the left inguinal region. Epidermis normal. The basal cells were hyperpigmented. There are slight perivascular round cell infiltrations in the superficial subcutis and the tumor tissues clearly circumscribed in the deeper subcutis. Excepting the stromas, which consist of thin connective tissures with vascular dilatation and slight infiltration of round and a few other cells, the tumor are filled with irregularly sized, partially poorly stainable round, oval, polygonal and fusiform cells. Their nuclei present various quantity of chromatin, the figures of necrobiosis and mitosis in some places. These tumor cells present mostly the fascicu-

lar arrangements, but the strict examinations show the alveolar arrangements in a few places. There are no melanin granules, no dopa positive cells and no oxygen area by Unna's permanganate-methylgreen method in any places. These findings seem to be like the spindle-cell sarcoma at a glance, but the fact that the larger prickle cells present alveolar arrangements in some places suggests the basalioma or carcinoma. We cannot decide the conclusion only from these findings.

3) Melanotic tumor on the left pectoral region. Epidermis is somewhat thinned. The basal cells are highly hyperpigmented. In the deeper corium, there are groups of fusiform, stellate, round and oval cells. Their situtations, morphologies and arrangements resemble to those of blue nevus. Nevertheless, we believe them to be the pigmented nevus cells. In the more deeper layer, spreading from the deepest corium to superficial subcutis, there are round and stellate cells which take the reticular or alveolar arrangements. These cells are so filled with melanin granules that their nuclei seem to be faded. In the more deeper layers, these cells change their arrangement gradually from alveolar to fascicular. From these findings, we know that the nevus cells change their arrangements with their situations. And then, we admitted that these nevus cell nests are the oxygen area by Unna's staining and present positive dopa reaction. But these two reactions are not so clear in the places where the cells are filled overflowingly with melanin granules.

4) Pinhead-sized lentigo on the brow. The basal cells are hyperpigmented. There are typical nevus cell nests in the superficial corium. These cells present positive dopa reaction. No malignant figure is found. We admitted that the nevus cells change their arrangements corresponding to their situation in this specimen too.

From abovementioned, we thought that congenital pigmented nevus was stimulated by the "Multin" injection and there occured Sutton's leucoderma in the local and nevocarcinoma in whole body. The melanotic tumor cells presented positive dopa reaction and oxygen area by Unna's staining. The amelanotic tumor cells, on the other hand, showed negative dopa reaction and no oxygen areas. These facts reminded us of Greenstein's[9] description. He believed that the melanotic melanomas, in contrast to the amelanotic melanomas, were characterized by the possession of 1) cyanide-sensitive tyrosinase, 2) cyanide-sensitive dopa oxydase, and 3) enzymatic system, or systems, which oxidizes *p*-phenylendiamine and which is largely insensitive to cyanide. And Cailliau[10] attached great importance to *p*H in the field of the melanogenesis. From above we conclude as follows: the existence of amelanotic tumors does not always signify that the cases are taking very malignant course as some authors have believed, but it shows that the biochemical conditions in their fields, especially oxidoreduction potentialities, are insufficent for the pigment production.

Malignant Melanoma on the Sole

The melanoma is not commonly found in the darker races, but there are some reports of the cases on the sole where the pigmentation in the skin is very scarce (Bishop). In Japan, T. Ito (1906) reported a patient, aged 56, of malignant melanoma on the sole and since that time many authors described similar cases: Sekiba (47 y. female, 61 y. female), Ono (53 y. male), Kawanishi (66 y. male), Miyata (47 y. female, 58 y. male, 38 y. male), Uchida and Nokata (67 y. male, 56 y. male), Watanabe (67

y. male) and Ninomiya (67 y. male). We experienced similar case racently.

Case Rreport: A man, aged 67. First examination in 1947. His mother died of gastrocarcinoma. About one year ago, he noticed that a redbean-sized red spot grew on his right sole with no conscious cause. After two months, that spot broke down naturally and showed yellowish secrete. Then the bulla reappeared on the same place and soon light-yellowish tumor developed from the bottom of the bulla. This tumor enlarged gradually and became plum-sized presenting black tone. The patient came to our clinic complaining of the pain by walking. Status presens : General inspection showed no abnormality. Blood sedimentation rate moderately accelerated. There is a plum-sized, black, roughly papillary tumor on the frontal-half of the plantar arch of the right sole (Plate XI, Figure 43). The dirty, greyish white purulent secrete covers the elastic hard tumor which is limited sharply from the surrounding skin with red inflammatory halo. Right, one or two, hard femoral nodes can be palpated in fingertip-size. Course : Extirpations of the tumor and femoral nodes were performed and the patient took good progress. After about two years, he suffered from pleuritis exsudativa dextra and haemorrhagic effusion was removed several times by the punctures. He became cachexia gradually and died. From these facts, we think there occured the metastasis of the cancer. But he showed no melanuria throughout his progress.

Histological and histochemical findings: The major of elevated part of the tumor is the thickest stratum corneum, which contains many melanin granules everywhere. And the layers, situated immediately beneath the stratum corneum, consist of the nucleated cells with the homogeneous protoplasm. The stratum granulosum is scarcely found. As there are no intercellular spaces in the superficial prickle-cell layer, the cells are united closly each other and translate to the upper parakeratosis-like cell layer. The intercellular spaces and processes are slightly found in the deeper prickle-cell layer. The basal-cell layer is very irregular and shows figures of "thèque (Darier)" in some places (Plate XI, Figure 44). There are the cells with clear protoplasm or with vacuolization, which contain no melanin granules, and the flattened cells which contain melanin granules. The epidermal processes invade in the irregular form or drop down into the corium in the form of "Zellregen." When they show a transverse section, they present cancroidlike figure. These findings correspond with "Melanoma parakeratodes (Merenlender)." In the corium, the groups of polygonal or stellate cells show the alveolar arrangement. They have the great oval nucleus with the scanty chromatin and the melanin granules containing protoplasm which shows in mostly the vacuolization. Some of thes figures correspond with socalled "ségrégation (Darier)." In the other field, however, these cells have the pyknotic nucleus and show the syncytial figure. Everywhere we find mitotic figures. These melanin containing cells take alveolar, reticular and fascicular arrangements. These arrangements transform their figures by their location in cutis : alveolar in the superficial corium, reticular in the more deeper and fascicular in the deepest (Plate XI, Figure 45). Thus the coexistence of "cellule sphérique" and "cellule rameuse" (Masson) is admitted. In other words, "mélanome endocrinien achromique" and "Spindelzellensarcom" are found in the same specimen. These findings correspond with "mélanoma dimorphe (Masson)." Examining these pigment cell nests by Mallory's staining, no fresh bluish fibril penetrates into them. Sudan III and Nile blue stainings show the lipoids, which

are double refractive by Nicol's apparatus, in the tumor-cell nests. (These points correspond with the observation by Kreibich.) From these facts, we know that these lipoids are cholesterins and the protoplasm which seemed to be vacuolar contained these substances. And then, by Unna's permanganate-methylgreen method, the tumor cells are stained intensly with methylgreen corresponding to the quantities of their containing melanin. From this, we know that there occurs the intense oxidation in these tumor cells. In the stroma of the corium, there are found generally the loose tissue where the slight round cell infiltration acompanies with a few plasma cells. The chromatophores increase in number at the places attached to nevus cell nests. From above findings, we diagnose this case as melanocarcinoma.

Melanoma on the Lips

Since Weber (1859), it has been admitted that when the melanomas were found on the buccal mucosa, they were seen most frequently on the palate. For examples, New (1929) collected 25 cases of melanoepitheloma on the palate at Mayo-Clinic and Fuhs & Kumer[11] collected 31 cases of melanosarcoma on the buccal mucosa from the literature, which were originated, without exception, from the palate and the maxillary dental alveolus. In Japan there are the reports by Kudo, Tsutsui, Nikaido, Kajitani, Tsuzuki and Iio.. The cases on the lip were reported by Ono (55 y. male, 1918) and Takahashi (63 y. male, 1926). We experienced recently a case on the lip.

Case Report: A woman, aged 59, first came to our clinic in 1947. Her mother, six brothers and sisters and two daughters showed the ephelides. But other family history presents no abnormality. About fifteen years ago, she remarked a pinhead-sized black spot on the right side of the upper lip. But she took it for the ephelides and did not received any treatment. After three or four years from that time, the spot increased in size and invaded into the red lip. Since January, 1947, she suffered from the bleeding when opens her mouth widely to take meals. Status presens: Build and nutrition normal. There are freckles on the face, forearms and trunk and the senile atrophy of the skin proper for her age. A jet-black spot on the right side of her upper lip distributed from the median line to the right labial commisure, taking its center in the red lip (Plate XI, Figure 46). It spreads to the skin over the upper lip in irregular form, where it presents more lighter browish tone than that of red lip. On the other side, the black spot extends on the mucous membrane inside the lip and shows the deep bluish color at its edge. The gingiva and the surface of the right second incisor present the deep black tone which don't fade even by severe rubbing (melanodontia). The boundary is very clear but slightly zigzag. At the red lip, this spot takes the papillary form and is covered by greyish white squama which can be stripped forcibly only with slight bleeding. The palpation shows somewhat thickness on these parts but no infiltration in the deeper place. She don't complain the itching. Only a left neck lymphatic gland can be palpated in pea-size. Blood pressure low for her age. Blood sedimentation rate slightly accelerated (56–82). Course: Radiumtherapy was repeated four times with intervals of a month. The pigmentation was faded partially and the bleeding in the rhagadic places became better. After half year, when she came to our clinic, the tumor presented enlarging tendency. So the repeating radium therapy is being carried on.

Histological findings: The epidermal thickness is very different by places. No hyperkeratosis. In some places, however, the melanin granules arrange themselves like the parakeratous nuclei. The stratum granulosum consists of five or six layers. In the deeper prickle-cell layer, there are many cells whice involve melanin granules around their nuclei. The basal cells, consisted irregularly of cylindric and round cells, contain many melanin granules and, in some places, present the vacuolizations (Plate XI, Figure 47). The epidermal processes invade into the cutis very irregularly and present the figure of "Zellregen" in some places. In the corium, from the papillary to the reticular layer, there are round and stellate cells, whose protoplasms take so many melanin granules that their nuclei seem to be faded, in the alveolar or fascicular arrangements. From these findings, we know that these cells are the nevus cells. The changes in the stroma are considerable: dilated blood vessels, slight bleedings, infiltration of the round-, plasma cells, histiocytes and fibroblasts, and the chromatophores increased in number among abovementioned cells. Thus the findings are very complicated. We diagnose this case as melanocarcinoma nevogenes originated tardily from the ephelides or the abortive type of xeroderma pigmentosum.

Melanoma on the Pudenda

The melanomas on the pudenda are found more frequently in woman than in man. We experienced recently a case complicated with carcinoma on the labia pudendi.

Case Report: A woman, aged 53, came first to our clinic in 1946. She was operated twice, in 1929 and 1932, with the provisional diagnosis of uteruscarcinoma and became amenorrhoea since that time. In 1938, she was operated for the tumor on the anal region and the swelled right inguinal glands. Since January, 1938, she remarked a little-fignertip-sized, dark red tumor on the right labia majora with stiching pain. Salvarsan injections brought about no improvement. The tumor and the right inguinal glands enlarged more and more. Status presens: Blood Wassermann's reaction (-), blood sedimentation rate (83–102). There is a cockscomb-shaped tumor on the right labia majora and its surface is papillary, macerated greyish or ulcerated with light red tone. It is covered generally by the purulent secrete and very stinking. On the anal region, a dark red hemorrhoidlike tumor is found. They are palpated elastic hard as whole. She complains a slight pain by compression. The border is considerably sharp. Several, hard, right inguinal glands are palpated in thumbtip- to pigeonegg-size. On the left labia majora, no swelling and infiltration are found. When the tumor on the right labia majora is compressed to outside, the atrophic, hard vaginal orificium and wall can be palpated and the latter is ulcerated painfully in some places. Near to the left commissura labiorum posterior, a halfring-shaped, bluish black, narrowly elevated region is palpated somewhat hard (Plate XII, Figure 48). The skin in this region presents the lightred tone and is thickened slightly, but there are no infiltration in its deeper part and no pain by compression. Course: After about a week from the extirpation of the tumor on the right labia majora, the halfring-shaped melanoma on the left side lost its black tone. This tumor was extirpated and observed microscopically. Since that time, there was favorable progress for short time, but the relapse occurred soon. The extirpation, X-ray- and radium-therapy

were performed energically for about eight months. But there is no favorable effect and the patient was discharged. Her family reported her death soon after that time.

Histological findings: The tumor on the right labia majora presents a typical figure of basal-cell carcinoma and, in some places, Bowen's socalled " clumpings cells " are found. The suddenly disapeared tumor on the left side presents next findings. Epidermis very thickened, a few epidermal processes found, and horny layers not so clear with one or two parakeratotic layers. Attaching to two or three granular layers, there are the prickle cells, which so lose their prickles that they are close each other. They are not so good stainable and their protoplasms show the vacuolizations everywhere. In the deeper prickle layer, there are larger, round, chromatin-rich nuclei. They became gradually like the basal cell and finally they transit to the basal-cell layer, which consists of cylindric palisade cells and which presents no abnormal change such as vacuolization. These findings correspond with those of the basalioma. In some places, however, the cells with fusiform nuclei and gigant cells as in Bowen's disease are found. This finding corresponds with that of the tumor on the right labia majora. The basal cells involve a few melanin granules, but some cells in the superficial Malpighian layer involve the dendritic or massive melanin granules. In the corium, a few chromatophores are found. Thus, there is no figure of melanoma.

The prognosis of melanoma is very various and there are some reports showing the natural cure (Ribbert, Borst, Cange and Deboucher). Bertier-Weissenbach[13] and Darier[14] reported the cases in which the metastatic tumors bacame smaller and finally disappeared by the extirpation of the chief tumors. We believe this patient as similar case.

Panaris Mélaniques

Melanomas on the fingertips or the nailbed were observed first by Hutchinson in 1881 and the French school, Dubreuilh and Darier, named them " panaris mélaniques " or " tourniole mélanique " as specific type. Since that time, Specht and Chauvenet-Dubreuilh collected similar cases from the literature. Adair, Pack, and Nicholson[15] at Memorial Hospital counted 26 cases of the subungual melanoma out of 218 cases of melanoma. Womack,[16] taking his materials from the patients of Barnes Hospital in St. Louis, reported that this disease was found in 15% of all melanomas. The cases in the Black races were reported by Adair-Pack, Bauer-Dickson and Jarman. In Japan, since the first report by Kumagai in 1906 (62 y. female), Inoue (51 y. female), Kawamura (57 y. female), Miyata (51 y. female) and Yano-Nohara (26 y. male) reported their cases as the melanoma on the fingertip. These cases occured frequently in over fifty years of age, in women and especially on the thumbs. From these points, it has been thought that the traumas play a great rôle as the accelerating causes.

Case 1.—A woman, aged 60. First examination in 1942. Her elder sister died of gastrocarcinoma, but no other family history. Since three years, she remarked a red-bean-sized lentigolike tumor on the nail side border of left thumb. Spreading to the volar side of the thumb, it enlarged gradually and became easy to bleed. As the nail side border suppurated with pain about a month before, the incision was performed. Since that time, the tumor added the speed of its enlargement. Status

presens: Healthy woman at first sight. The swelling of the aorta and hypertension were found, but blood Wassermann's reaction was negative. Slight albuminuria but no melanuria. There are excoriated, irregular-sized, bluish black spot, taking its center in the nailbed of the left thumbtip and extending from the dorsal to the volar side (Plate XII, Figure 49). This looks as if an Indian-ink permeates under the epidermis. These places are slightly elevated and bordered clearly from the circumference. One or two pustules are found on the nail side border. The nail plate, which is thickend markedly and loses its polish, shows deep black longitudinal bands on both sides (melanonychia), and retains its natural color only in the center. But there is admitted no infiltration, only slight roughness in all places. She complains of pain by moving the nail-plate. A plum-sized enlarged node is palpable with slight pain in the left axillar region.

Histological and histochemical findings: Stratum corneum and rete Malpighii thicken highly corresponding to the location of the fingertip. At the center of the lesion, there are fusiforme, nonnuclear, pigment granules containing cells, which are circumscribed in some places and which are localized horizontaly as fish scales. But no parakeratosis is found. Stratum granulosum consists of four or five layers. The prickle cells show the perinuclear vacuoles everywhere. The basal cells, which show the irregular form and arrangements, change themselves into foamy, vacuolar form at the top of the eidermal processes. And, attaching to them, there is the figure of "Akantholyse." Moreover, there are some basal cells, which dropped off in the figure of socalled "Zellregen" attaching to the epidermis or separating in the cutis. The latter cells, surrounded by the connective tissue, present the nests of round or stellate pigmentcells. Especially in the little nests, we admitted the figure of so-called "cellules claires (Masson)" or "Zwillingszelle (Merkel-Ranvier)." The chief change in the corium is the remarkable increase of the pigmented cell groups, which invade into the papilla from the subpapilla and whose pigment granules are grosser and show intense argentaffinity (Plate XII, Figure 50). These cells present the fusiform, stellate or round form and are filled with so much pigment granules, that their nuclei cannot be found. These pigments are negative in the iron-reaction, fade slightly by the 24 hours' procedure of H_2O_2 and lose the black color almost completely by 48 hours' procedure. From these facts, they are admitted as melanin granules. These pigment cells are found in groups around the capillaries or the nests of the epidermal cells, which are migrated from the epidermis to the corium. But their arrangements don't always show the alveolar structures as in the nevus cell nests, where the melanin granules are rich in the peripheries but a few in the centers. According to Bielschowsky-Maresch's staining (Plate XII, Figure 51), the pigmentcells are encapsulated by the argentaffin fibers and the close relationships between them and the argentaffin fibers around the capillaries in the center can be admitted. But these argentaffin fibers don't invade into the places, where the vacuolar changes are presented in the top of the epidermal processes, and into the epidermal cells dropped off into the corium. The capillary-intimas in the papilla, where the pigmentcell infiltrations are found, are thickened so highly, that their spaces become very narrow and the subpapillary blood vesseles are dilated and filled with blood cells. Everywhere the moderate infiltrations of little round cells and histiocytes are found. The nerve fiber staining by Bielschowsky-Seto's method shows that there are rich in the nerve organs corresponding to this

location, fingertip. Wagner-Meissner's, Vater-Paccini's corpuscles and many nerve fibers are admitted. Many thicker nerve fibers and fine vegetative nerve fibers, which intertwine with the blood vessels, penetrate the pigmentcell infiltrations. Moreover, the periphery nerve fibers invading into the epidermis from the papilla are found everywhere. And then, some of nerve fibers in the stratum reticulare are thickened fusiform or braid-like in shape and some of them in the deeper reticular layer present a glomerular form, so that they are admitted as the little nest of neurino-fibroma. But there are no changes anywhere as "corpuscules naeviques" and "lames foliacées," on which Masson based his neuroectodermal theory of nevus cell. Examining the relationships between these nerve fibers and the pigmentcells, we get next findings: Meissner's corpuscules are encapsulated with the pigmentcells but no argentaffin granules are found in the corpuscules, and, around the transverse section of nerve bundles in the stratum reticulare, there are fusiform pigmentcells but they don't invade into the nerve fiber sheaths.

Case 2.—A woman, aged 46, had a trauma on the left thumb about five years ago. This region was suppurated about two years ago. Repeated incisions brought about no improvement. On examination in Jan., 1951 she showed socalled panaris melaniques on the nail and endophalangeal region of left thumb and some symptoms of mental disturbance. She died in Oct., 1951. The autopsy presented the metastasis of the melanoma on the cerebrum, ribs, omentum majus, gallbladder, stomach and ileum. In the specimen obtained from the volar side of left thumb, typical nevus cell nests could be found. Therefore we diagnosed this case as melanocarcinoma. (The details will be reported another day in collaboration with Yamamura, department of psychiatry, Hirosaki University.)

Milian[17] pointed out the fact that there coexist both histological findings of melanocarcinoma and melanosarcoma. Our first case seems to present such findings as Milian described. But we could diagnose distinctly the second case as nevocarcinoma. We believe that the majority of panaris mélaniques is melanocarcinoma, though their histological diagnosis is very difficult sometimes.

Mélanome Malin Mésenchymateux or Melanosarcoma

In 1925, Darier[18] described three cases of malignant melanoma originated from the blue nevus. This disease is very rare and, inspite of its designation (melanoma), it is not tumorous but the bluish or slate-blue flat spot, in which the black or deep brown shrapnel-like nodules are dotted and somewhat roughly palpated. It malignizes suddenly in the puberty. The locations in Darier's three cases, were auricular, mandibular, neck, scapular and pectoral regions. The regional lymph nodes swelled as in inflammatory changes, but when they presented the metastasis-like changes, the patients took frequently the unfavorable prognosis. The progress, however, are gradual in many cases and some of them are cured with electrocoagulation. And so we cannot conclude all of them to be malignant.

Histological findings: The fusiform, stellate and long fibrous cells involving the pigment granules are found in group among the connective tissures or around the blood vessels in the deeper corium as in blue nevus or Mongolian spot. From these findings, we think that this is just mesenchymal melanoma and must be dis-

tinguished from the nevogeneous melanoma as described above.

But many cases reported as melanosarcoma in Japan, we believe, are not always limited strictly in the mélanome mésenchymateux in the sense of Darier. For example, the malignant melanoma originated from the fasciculated form of nevus cell has been described as melanosarcoma. On the other hand, since Stranz,[19] the cases, in which the blue nevus and the pigmented nevus were complicated, have been reported. Recently Kawamura[20] described also a similar case in Japan. When these cases become malignant, the histological findings, we believe, become more and more complex. We know a good example of these cases. That is a case reported by Ozaki[4] at the pathological labolatory of our University in 1933. The case is as follows. A man, aged 52, presented an extensive blue nevus which spread on the right forehead, temporal and parietal regions and which was complicated with the shrapnel-like black nodules. Showing generalized metastasis and melanuria, it took malignant progress and he died. The biopsy was performed. Ozaki, from the point of Ribbert's chromatophore theory, diagnosed his case as melanosarocoma. We could examine this preparations by the kindness of the pathological laboratory and we found that there coexist both typical findings of the blue nevus and the malignized pigmented nevus with the alveolar or fascicular arrangements in the original location on the skin. From these findings, we cannot agree to make the case as melanosarcoma. We think it proper to diagnose the case as the complicated form of the blue nevus and the melanocarcinoma. To say the least, this case does not correspond to the melanosarcoma in the Darier's sense.

Summary

From abovementioned, we conclude as follows:

The malignant melanoma, whose majority had been considered as the melanosarcoma by many authors, is melanocarcinoma with a few exceptions.

The malignant melanoma originated from the blue nevus is melanosarcoma as Darier described. But we must not forget the facts that the nevus cells change their arrangements from alveolar to reticular or fascicular corresponding to their situations and that some cases show the coexistence of pigmented nevus and blue nevus. When these cases became malignant, the strict examinations are necessary for their diagnosis.

The existence of amelanotic tumors in the malignant melanoma does not always signify that the cases are taking very malignant course, but it shows that the biochemical conditions in their fields, especially oxidoreduction potentiality, are insufficient for the pigment production.

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XIII. Vitiligo*

Vitiligo is one of the common dermatoses, the percentage of which to all ambulatory patients in dermatological clinic is 1.9 in Kanazawa and 1.3 in Sendai. The etiology and pathogenesis, however, have not been thoroughly understood. Amongst various hypotheses, the theory of nervous disturbance is most widely accepted. It is well known that in some cases the site of eruption shows the tendency to follow the distribution of cutaneous nerve, and the observations of Sasamoto[1] and Takikawa[2] seem to support this view. Moreover the socalled pigment lability of the individuals induced by dysharmony of autonomic nervous system and disturbance of endocrine function is admitted to play a rôle in the etiology. The author has already presented several observations suggestive of the relationship between pigment production and nervous system. In the following I will first discuss the etiology of leucoderma centrifugum acquisitum Sutton, in which the participation of nervous system is most probable. Such a study, I believe, will throw some light on the dark etiology of vitiligo, too.

1. Sutton's disease,[3] the identity of which with vitiligo is still under discussion, is not extremely rare. In the recent 10 years we observed 9 cases including 2 atypical ones (Table XIII). The numbers of vitiligo during this period was 264. In most cases of Sutton's disease, pigmented nevus in form of lentigo is situated at the center of the lesion. In some, however, hairy (Birger, Stokes, Krantz), papillary or usual pigmented nevi (Fuse, Arakawa) are noted, and rarely fibroma (Leszczynski, Mgebrow and Pridsky, Richter), angioma (Rabut), verruca senilis (Petges, Reito, Szenthiralyi), psoriasis, sarcoid (Stokes), lichen planus (Robinson), scar tissue

TABLE XIII

Case number	Age in years	Sex	Location	The lesion in the center of the patch	Concomitant vitiligo
1	19	F	Right forehead and right mandibula	Lentigo	(−)
2	29	M	Left nape	"	(+)
3	31	M	Left neck	"	(+)
4	38	M	Back (two patches)	"	(+)
5	23	M	Right back	"	(+)
6	12	M	Right back	"	(+)
7	20	F	Chest	"	(+)
8	42	M	Abdomen	Papillomatous pigmented nevus	(+)
9	52	F	Left chest and right back	Keloid	(+)

* By Minor Itô.

(Netherton and Curtis) or erythema (Mgebrow and Pridsky) are reported in place of the mole. The latter condition is beyond the category of vitiligo périnaevique.

In discussing the etiology of Sutton's leucoderma, it must first be made clear whether the lentigo appears first or the leucoderma. It is difficult to solve this problem dicisively because such trifle lesion as lentigo is likely to be overlooked and thus the statement of the patient cannot be accepted as acurate. Sutton, Weber, Richter, Shengsum, Hebijima[4] state that vitiligo is the primary eruption, while Stokes, Póor, Rostenberg, Gougerot, Sasamoto, Tosima, Kodama, Higuchi-Boku[5] considered the depigmentation as secondary. Leszczynski, Reito, Mori hold eclectic opinion that leucoderma and mole are co-ordinated in clinical occurrence. Sutton in the histological study looked upon the hyperpigmentation of epidermis and the endothelial cellular infiltration in the corium in the central part as secondary changes following vitiligo. His theory was accepted by Schelmire and Bunch. Petges, Stokes, Casseberry found that what Sutton called endothel cell is no other than nevus cell, and Toyama, Goldhardt, Póor, Mgebrow-Pridsky accepted that the central lesion is true pigmented nevus. These findings lead to the conclusion that pigmented nevus is the primary eruption and thus the conception of vitiligo périnaevique is established. The author also noticed nevus cell in the histological study (Plate XIII, Figure 54) and is convinced of the initiality of nevus lesion.

The relationship between leucoderma Sutton and vitiligo is much discussed. Some authorities tried to take leucoderma Sutton as a distinct disease from vitiligo by following observation: the existence of cases in which vitiligo is not co-existent, incidence of spontaneous recovery, successful treatment with ultraviolet irradiation, differences in histological findings. These, however, cannot be definitive characteristics. For instance, most cases are combined with vitiligo (in my experience 8 out of 9), in some cases halo is noticed, extension over 10 cm in diameter is observed. Successful treatment with ultraviolet irradiation or spontaneous recovery can occur in vitiligo. And histological findings differ by cases. In my comparative study, transitory picture is obtained. These findings, in common with the results of dopa reaction, show that these two dermatoses are most likely to be identical.

The author holds to this conception and further tries to investigate the etiology of vitiligo from this standpoint. In my previous report[6] I accepted Masson's neurogenic theory of nevus cells and at the same time assumed that melanin production is influenced by the chemical transmission at the termination of vegetative nerve fibrils. Consequently the congenital abnormality in the distribution of termination of nerve fibrils at the site of nevus lesion is surmised. And the depigmentation of leucoderma Sutton may be caused by the abnormality of vegetative nervous system accompanying central nevus lesion in the sense of trophoneurotic disturbance. Judging from abovementioned considerations it may not be too eccentric to surmise the etiology of vitiligo and leucoderma Sutton in the following manner: in vitiligo the disturbance is found in the neighbourhood of spinal ganglion and hence the symmetrical or unilateral systematized arrangement while in Sutton's disease the nervous disturbance limited in the area of nevus results in the circumscribed vitiligo périnaevique with only little tendency to extension.

2. Following cases of my recent observation are in favor of neurogenic theory.

a) 55 years old woman with vitiligo of the back for three months duration. On

the lumbar region and in the middle line several dark brown islets are observed at regular intervals as if the area above the spinal ganglion are left intact (Plate XIII, Figure 52).

b) 50 years old man. Depigmented spots which developed recently appeared symmetrically on both sides of the spine (Plate XIII, Figure 53).

c) 3 cases of vitiligo in which the lesions are arranged symmetrically beginning from both sides of underlip to the sides of neck just like the predilection of bilateral herpes zoster of this area.

3. It is not rare to find vitiligious lesion with normal colored mamilla in the center. I have observed 8 such cases. As mamilla is physiologically rich in melanin and, moreover, is possessed of special nerve distribution, we can assume that these phenomena are closely related to Sutton's leucoderma. The absence of muscular reflex in vitiliginous mamilla (Levy-Frankel and Juster)[7] is in favor of this conception.

4. The rôle of vasomotor nerve in the etiology is suggested by following observations. In the histological study of vitiligo as well as leucoderma Sutton, dilation of blood vessels and perivascular cellular infiltration are often noticed in the papillary, subpapillary layer in the neighbouring zone of depigmented area (Plate XIII, Figure 55). This, in accord with occasionally perceptible erythema on the perivitiliginous area, and the érythème prévitiligineux of Milian, Maslot and Horowitz[8] suggests the abnormality of vasomotor nerve. The abnormal cutaneous reagibility against various agents (carbon dioxide snow, canthalis plaster, epinephrine, morphine, caffeine, tuberculine, diphtheria toxin, myoarsphenamine, phenolphthaleine) found by Takikawa may support this theory.

5. In cases of vitiligo and Sutton's leucoderma, disturbance of sensibility is usually lacking. But in rare instances itching or hypesthesia is complained (Königstein, Thibierge, Sasamoto). In such cases it may be assumed that the disturbance of vegetative nervous system, which I already pointed out, may have extended to sensible nerve fiber which runs usually accompanied with vegetative nerve fibrils. Further evidence suggestive of nervous disturbance is the occurrence of vitiligo in tabes or following injuries of brain or peripheral nerve, and the fact that nerve leprosy is frequently accompanied with depigmentation.

Leloir and Chabrier, Dejerne,[9] Marc[10] payed attention to nerve atrophy in form of myelin fragmentation, increased Schwannian nuclei in the histological study. Darier, however, was sceptical about these findings. The author stained sections obtained from vitiligo and leucoderma Sutton with Seto's modofication of Bielschowsky's silver impregnation method. In thick nerve bundles (sensible nerve), hypertrophy and irregularity in thickness of axis cylinder and increase of Schwannian nuclei are observed. However, neuritis degenerativa atrophica is not ascertainable. Terminal reticulum of vegetative nerve fibrils, clearly stained in each section, is apparently normal. Naturally it is impossible to draw certain conclusion as to the functional state of nervous systme solely from the results of silver impregnation method which does not always give constant results.

6. We Itô and Watanabe[11] examined local blood of vitiliginous area. The differential count and oxydase reaction are almost the same as control peripheral blood. Peroxydase reaction, however, is markedly weaker. And, moreover, the results of oxidoreduction staining of Unna suggest specific local characteristics

especially in reducing activity of the site. This may have certain relationship with the increased content of sugar and iodic acid reducing substance of canthalis blister fluid at the vitiliginous area (Takikawa[12]).

Treatment

No definitive therapeutic measure is available as yet, and only various endocrine preparations and ultraviolet irradiation either by itself or in combination with photocatalytic substances are tried at present. We have been studying the pigment problem partly for the purpose of obtaining definitive treatment of vitiligo. In animal experiment (Itô & Nakajo[13]), it is possible to produce black pigment in melanin free goldfish which has same chemical property as natural melanin by injection of *p*-benzoquinone. This substance, however, has no therapeutic effect in human vitiligo. And the same for tyrosine, tryptophane and adrenalin. Itô and Watanabe[14] further examined the effect of several endocrine preparations and biologic active substances on melanin free goldfish. Among substances examined, only hematoporphyrine and natural anterior pituitary hormone can produce black pigment which is maintained for relatively long period. In clinical application, both substances are injected intracutaneously in the vitiliginous area with or without following ultraviolet irradiation. Each injection is given at several days' interval when the local inflammatory changes have subsided. No side reactions are noticed except for slight porphyrinuria in cases of long continued porphyrine therapy. Usually after 3–4 months treatment, tiny pigment flecks are newly formed which by enlargement and confluence substitute the entire lesion. At the same time, hyperpigmented halo becomes more intensely pigmented. Histological examination of newly formed pigment spots gave following results. Pigment granules are contained in the basal layer and the dopa reaction is turned to positive. This newly formed pigment is breached by hydrogen peroxide procedure and is identified with natural melanin. This procedure, though rather time-consuming and expensive is superior to other type of treatment in therapeutic results. Then, how is the mechanism of pigment reproduction by these preparation is explained? Dietel, Zondek and many other authorities observed that hormones of intermediary and posterior pituitary influence color change in animals and matrimony lobe in fishes. Consequently pituitary preparations are often applied in the treatment of vitiligo (Fournier, Cerenino, Hayashi, Namiki). And Bloch[15] observed hormone of ovarian follicle which is secreted under the influence of anterior hypophysis, produced increased pigmentation on the nipple of guinea-pig. In our experience neither the preparation of posterior pituitary nor urinary gonadotropic hormone of anterior hypophysis are effective in producing pigment in goldfish. On the contrary, the extract of anterior hypophysis showed some effect. This is of interest in view of the fact that only this preparation showed positive melanophore reaction. So it may be that some or other hormone contained in the extract of anterior hypophysis may act as hormozyme (Euler) in melanogenesis, or may activate dopa oxydase, in the sense of neurohormone of Parker and Vilter.[16]

Hematoporphyrine is a well known photocatalyser. In our experiment with goldfish, melanin production was noticed solely on the back where the effect of ultraviolet ray was strongest. Therapeutic result in vitiligo was most markable in area

treated in combination with ultraviolet irradiation. Thus the effect of hematoporphyrine is attributed to photodynamic action on the contrary to that of anterior pituitary hormone.

Summary

In the comment on the pathogenesis of vitiligo, nervous disturbance theory was accepted as most probable especially through the study of leucoderma Sutton which the author looks upon as a variety of vitiligo.

Several cases of vitiligo are presented in which the participation of nervous system was surmised.

No definitive therapeutic measure is available as yet. However, intracutaneous injection of anterior pituitary preparation and combined hematoporphyrine ultraviolet irradiation method gave superior result to other treatment.

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XIV. Dyschromatosis Symmetrica on the Extremities*

The clinical manifestations of pigmentary disturbance are far more complicated in our race than in the White and the Negro races. So we often observe such diseases as are not reported in foreign literatures. Dychromatosis symmetrica hereditaria is a specimen in point. This disease was reported independently by Komaya,[1] Matsumoto[2] and by Toyama[3] under three different designations. The characteristic cutaneous change consists of small pigment flecks resembling freckles mingled with reticulated depigmentation disseminated on the face and at the ends of extremities (Plate XIV, Figure 56). It is differentiated from freckles because of the co-existence of depigmentation and from xeroderma pigmentosum because of the lack of erythema and atrophy. However, the independence of this disease is not well established. For instance, the photosensitivity in xeroderma pigmentosum varies in a wide range according to cases and the clinical manifestation of hyper- and depigmentation differs according to the physiological color tone of the skin in individual race. So the lack of apparent photosensitivity and co-existence of hyper- and depigmentation cannot be always intertreted as pathognomonic feature of dyschromatosis symmetrica hereditaria. I[4] have described previously that this disease belongs to same category to that of freckles and xeroderma pigmentosum from the genetical point of view.

Pigmentary disturbances similar to dyschromatosis symmetrica hereditaria are noticed under several conditions.

1. Safu in Caroline islands is one of them (Plate XIV, Figure 57). Although the high incidence of heredity is noticed by Matsunaga,[5] it is difficult to exclude all postnatal factors such as framboesia and dermatophytosis (Melung of Ziemann for instance). Further dyschromatosis-like eruption following undoubtedly acquired disease is not so rarely observed in these islands.

2. Dyschromatosis symmetrica following pernio. It is not unusual to find depigmentation at the site of previous pernio eruption. However, the present author noticed that in small number of cases who suffer from pernio, symmetrical dyschromatosis-like lesion occurs chiefly on the dorsal surface of finger joints independently of pernio-scars. I have observed 13 such cases in the recent 10 years (Plate XIV, Figure 58). This, I assume, is resulted by the peripheral circulatory disturbance through coldness or by the reccurrent inflammation due to coldness. According to Kuré and Yamagata,[6] pernio is caused by trophic disturbance in hypotonic stage of parasympathicus. They thus recommended application of pilocarpine-lanoline ointment. Tomita[7] in my clinic measured temperature of skin surface and tried functional test of arterioles. His findings show that pernio is caused by disturbance of vasomotor nerve especially in the arteriole portion on the ground of vegetative nervous system. The author[8] obtained excellent therapeutic results in the topical application of camphor-lecithine-petrolatum ointment. This may

* By Minor Itô.

be explained by the assumption that lecithine which penetrated the skin is converted into choline or acetylcholine and thus exerts stimulation to parasympathicus. These findings disclose the labile autonomic nervous system of pernio patients, and thus I think that the pigmentary distrubance predisposes to these patients.

3. Dyschromatosis symmetrica following erythromelalgia.[9] In a 42 years old farmer who had suffered from erythromelalgia caused by mushroom poisoning *Clitocybe acromelalga* appeared a half year later irregular depigmented patches mingled with reticulated pigment spots on the back of both hands. The etiology of erythromelalgia is not entirely cleared. In Japan most of cases are caused by *clitocybe* intoxication. And it is generally admitted that vagotonic individuals are predisposed.

Comment

Pernio and erythromelalgia are somewhat related in the etiology because both lesions may be interpreted as manifestations of vasomotor or trophoneurotic disturbance occurring at the ends of extremities. And it is interesting to find that in both conditions pigmentary disturbance is occasionally resulted. Müller[10] observed similar depigmentation on the back of hands in a case of amyotrophic lateralsclerosis. And I[11] observed a woman, aged 61, of dyschromatosis symmetrica hereditaria in which the consanguinity of parents is stated. Since childhood she suffered from pernio and asthma, and after climacterium acroparesthesia appeared. Such cases are in favor of my theory because it is proper to assume that all these complaints developed on the ground of instable constitution of vegetative nervous system.

Summary

In the comment of the etiology of dyschromatosis symmetrica hereditaria, Safu and dyschromatosis symmetrica following pernio or erythromelalgia, the role played by the constitutional lability of autonomic nervous system cannot be overlooked.

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XV. Riehl's Melanosis*

Owing to the abnormal conditions of life under the influence of World War II, it is quite naturally expected that unusual dermatoses are to be seen. What I call Riehl's melanosis in this article is a specimen in point. Towards the end of the war and after war period, it is not seldom to meet with cases showing slaty brown bluish pigmentation of the face quite distinct from chloasma either with or without follicular eruptions. Such incident is noticed not only in our clinic but by many other dermatologists in Japan. In the literature such cases are reported as Riehl's melanosis[1] or melanodermitis toxica.[2] But the identity of these two dermatoses is still under discussion. In this article similar cases are summarized under the title of Riehl's melanosis because this is the most popular designation. However, I do not agree with German authorities who define malanosis Riehl so narrowly.

Clinical symptoms: As it is too time consuming to make description of individual case, only the characteristic points are stated.

The number of the patient is 32. All but one are female cases. The age of patients is most frequently between 20 and 39 years, while according to foreign literatures middle aged women are often affected. Itching, erythema and swelling of the face are complained as prodroms. In some cases fits of erythema, swelling and desquamation are noticed and the pigmentation grew severer after each fit. Similar observation is also made by Joulia and others.[3]

As regarding to the localization of the eruption, temporal, preauricular and mental regions never fail to show pigmentation. Other parts of the face, retroauricular portion and the sides of the neck may be involved, and in a few number of cases cubital fossae are also affected. The skin color of the affected area varies from slaty bluish bown to dark brown (Plate XIV, Figure 59). The skin surface in some is coarse and rough while in other is rather smooth and looks like powdered. Typical lichen planus-like eruption is found by none. By first glance the pigmentation forms rather diffuse patches, but on closer observation is found in tiny patches arranged reticularly corresponding to the superficial network of blood vessels. In long standing cases slight atrophy is noticed. In one case tinea amiantacea-like eruption is observed on the scalp. The pigmentation lose its intensity at retroauricular region and diminish into tiny patches and finally extinguish on the borderline to the breast. In a few number of cases pigment patches on the buccal mucous membrane are noticed, but yellow discoloration of sclera is not seen.

Histopathology and histochemistry:—In six cases biopsy specimens were excised.

Specimen No. 1. 31 years old female patient complaining pigmentation on the face for two months. Epidermis is thinner than usual with shorter processes. Basal layer contains only small amount of pigment. In the papillary and in subpapillary layer, especially near hair follicles are pigment cells situated close to blood vessels.

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* By Susumu Iijima.

Melanin granules are seen also in reticulum cells. No inflammatory changes throughout dermal layer.

Specimen No. 2. Obtained from a 30 years old woman. Duration of pigmentation more than two years. Thin epidermis. No hyperkeratosis. Several intraepidermal vacuoles are found. Perivascular infiltrates in the upper dermal layer consist of small lymphocytes and histiocytic cells. Pigment cells are increased in number. Melanin granules are phagocyted in the reticulum cells, too. Elastic fiber is lessened in the infiltrated parts.

Specimen No. 3. Section obtained from a 37 years old female patient with pigmentation on the face for three years duration. Epidermis is slightly hyperkeratotic. Rete thickened. Basal layer is poor in pigment. In the corium, perivascular edema and infiltrate of round and histiocytic cells are noticed. These reach to the middle layer of corium. Pigment cells are unevenly distributed in the papillary and subpapillary layers.

Specimen No. 4. 29 years old female patient complaining intensive dark brown pigmetation with slight grade of erythema for two months. Epidermal cells are somewhat disordered with intraepidermal vacuole formation near follicular openings.

The pigment content of the basal layer is smaller than normal. Hair follicles are strongly dilated and filled with horny masses. In the corium moderate perivascular and perifollicular infiltrates consisting of lymphocytic and histiocytic cells are seen in the papillary layer, where numerous pigment cells are found especially in the vascular surroundings.

Specimen No. 5. 28 years old woman. Duration of pigmentary disturbance for four months. Rete cells are vacuolated at several places and loosely connected. Borderline to the corium not distinct. Dilation of follicular openings is remarkable. Here and there intraepidermal cell infiltration is noticed. Basal layer contains subnormal amount of melanin. Numerous pigment cells are situated in the papillary layer with inflammatory cells (Plate XV, Figure 60). These reach to the deeper layer of the corium along blood vessels.

Specimen No. 6. This was excised from a 54 years old woman. The cutaneous eruption consits of follicular keratotic papules and perifollicular pigmentation. Epidermis is nearly normal. Follicular openings are strongly dilated. Pigment content of the basal layer is rather increased. In the corium are hyperemia in the upper layer and small round cell infiltration in the surrounding of blood vessels seen, where melanin containing cells are noticed. Inflammatory changes are most remarkable in the neighbouring zone of hair follicles.

Brief summary of the histological findings is as follows. The epidermis shows various grade of inflammatory changes and these coincide with the clinical symptoms but not with the duration of the disease. The melanin content of the basal layer is uniformly decreased. In the cutis are perivascular lymphocytic and histiocytic infiltrations noticed. Pigment cells vary in number in cases, and their form coincides with usual chromatophores. Remarkable is the fact that in most of the cases histological changes are most prominent in the neighbourhood of hair follicles. The increase of melanin containing cells in the upper dermal layer reminds one of incontinentia pigmenti, and recently Doornink[1] used the designation incontinentia pigmenti in the sense of a pathological feature and not a disease.

In the specimen No. 3. I stained the section with dopa reagent and got unexpectedly positive results. The technic employed is the modification of Fujiwara,[5] and I found that after 90 minutes at 37° C. small pigment cells in the upper dermal layer undoubtedly darkened (Plate XV, Figure 61). As the free melanin granules in that layer are not stained black, it is obvious that it is not due to the adsorption of oxidated dopa. The dopa reaction of Bloch has been estimated to be an indicator of the melanogenetic activity of cells and in all reported cases of Riehl's melanosis the dopa reaction is always negative. So in order to confirm these results I stained the sections with peroxidase reagent and with permanganate-methylgreen method of Unna. The results are quite corresponding. Namely, with Okano's[6] method peroxydase positive granules are indicated in the protoplasm, and with Unna's method the site of pigmentation stained with methylgreen as oxygen area (Plate IV, Figure 17). So it is found out that the pigmented area has high oxidizing potency. Therefore these results show that at least in some cases the dermal pigment cells in Riehl's melanosis act as melanoblast. Recently Capelli[7] presented similar theory in the problem of melanin production.

Etiology and Pathogenesis: According to the conception of Vienna dermatologists all pigmentary disturbances, in which external irritants are suspected to play a rôle, are excluded from the category of Melanosis Riehl. Such theory is not accepted now a days because Arzt[8] himself observed 3 male cases with apparently external causations, and Carteaud's[9] case recovered by stopping the use of cosmetics. However, it is impossible to attribute such cases merely to external irritants. With regards to the etiology I will take up photosensitization through coarse cosmetics as external factor and hypovitaminosis as internal and as supplementary factor disturturbance of endocrine and autonomic nervous system. Most of our cases are women of indoor activities having no occupational contact with tar, pitch or lubricating oil. So it is naturally suspected that crude cosmetics might be the external irritant. Such findings were reported also by Kitamura.[10] In the analysis of the cosmetics used by patients I found in three of them admixture of lower hydrocarbons and moreover, all presented the flavor of bergamot oil. These two substances I will take up as external irritants. It is well admitted that the application of tar or oil on the cutaneous surface accelerates pigment production. If so, the occurrence of melanosis must be more frequent in our country. Therefore, the participation of another factor is readily surmised.

In the animal experiment of Itô and Nakajo,[11] the injection of ascorbic acid and riboflavin can inhibit hyperpigmentation following bergamot oil application and ultraviolet ray irradiation. And this action is explained by the inhibition of the penetration of bergamot oil by these vitamins. If so, in hypovitaminotic state the penetration of such substance is easier than normal. The vitamin C content of the skin was tested by the modification of Rotter's method. The discoloration time of indophenole dyes is longer in this disease than controls. So it is possible that the patient's skin is more susceptible to external irritants. The same can be said with riboflavin. Though the determination of riboflavin was not made, we found higher level of pyruvic acid in patient's blood serum, and consequently surmise the deficient state of vitamins of same group. On the other hand the decrease of ascorbic acid means the loss of inhibitory action to the process of melanin

production itself.

As other signs of vitamin deficiency are almost lacking in my cases, it is not true hypovitaminosis but a sort of paravitaminosis in the sense of Comel.[12]

The rôle of autonomic nervous system in melanogenesis is not touched here. I mention only the frequent positive Aschner's phenomenon and positive pharmacodynamic reaction to adrenalin and pilocarpine.

The participation of endocrine system especially of adrenals and ovaries in this disease was already mentioned by Rolba. The results of recent investigations are not uniform. Adrenal insufficiency is noticed by Spira,[13] hypofunction of thyroid by Carteaud, while Sirres[14] found no endocrine disturbances. So we must wait for more comprehensive data in the future, and the fluorine-poisoning theory of Spira, hypercupremia of Panlais[15] require further confirmation. The author refers only to the facts that in some cases the pigment patches were noticed on the buccal mucous membrane and that in such cases blood pressure was low.

According to the dopa theory of Bloch the pathogenesis of this dermatose is explained by the increased pigment production in the epidermis and by the dropping of melanin granules from the epidermis to the cutis. While on the other hand Meirowsky,[16] supported by the animal experiment of Lipschütz,[17] maintains that the pigmented cell in this disease is not chromatophore but melanoblast. In my above mentioned histochemical study the enhanced oxidizing potency of the pigmented area was actually ascertained and in one case even the positive dopa reaction was noticed. Therefore it is probable that at least in some cases melanin production takes place in the corium, in the socalled chromatophores. Such theory is partly supported by the recent findings of Capelli who think it possible that melanin, under some pathological condition, can be produced in tissues and cells which contain no specific enzymes.

Treatment: Various therapeutic procedures are reported in this therapy-resistent dermatose. By the repeated injections of ascorbic acid, adrenal preparation and epinephrine combined with external application of weak alcoholic solution of resorcinol, we observed improvement of cutaneous disorders in several cases. Kitamura recommended the injection of small amount of oxophenarsine. Recently Kawamura[18] reported rapid recovery by intramuscular injection of BAL. In France the use of H 365 is recommended by Grupper and others.[19] However, in judging the effect of some or other method we must always keep in mind that spontaneous recovery is always possible in this disease.

Summary

This disease occurs by the external application of various irritative, photosensitizing substances in individuals in hypovitaminotic states of vitamin C and B complex. And endocrine dysfunction and dysharmony of vegetative nervous system play supplementary rôle.

In the histochemical study the possibility of dermal pigment production is surmised.

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XVI. Influence of Bergamotoil applied together with Vitamin C on Melanin Production*

In 1937 Urbach and Kral[1] reported an interesting observation that the melanin production is inhibited when bergamot oil, which is known as the substance increasing cutaneous pigment, is applied with vitamin C which inhibits the dopa reaction. The authors attempted the follow-up study on this mechanism in human and animal experiments.

Method: The flexor surface of the forearm of an adult is rubbed with alcohol then the agents are rubbed for five minutes and are rubbed off with alcohol after half an hour. The irradiation is made using quartz mercury vapor lamp (the shortest wave length is 290 mμ) with the uviol glass filter.

Experiment with bergamot oil and analogous volatil oils or saturated and unsaturated fatty acids: Of the agents examined including bergamot oil, volatile oleum lavandulae and terebinthine, saturated stearic and palmitic acid, unsaturated carboxylic and linolenic acid, none but bergamot oil increased pigmentation following ultraviolet irradiation.

Experiment with several irritants: As the result of applying 50% glycerin alcohol, 2% chrysarobin chloroform and coal tar and irradiating ultraviolet ray, all of them increased melanin production.

Experiment with additional vitamin C: The result which is showed in the Table XIV was obtained by intravenous injection of vitamin C (2 cc. contains 100 mg. ascorbic acid), applying each substance after half an hour and irradiating ultraviolet ray after 30 or 60 minutes. That is, only the result with bergamot oil was accordant with that of Urbach and in other applications the results were contrary (Table XIV).

TABLE XIV

Pigmentation after the Application of Pigment-increasing Substances and Ultraviolet Irradiation Following Vitamin C Injection

Substances topically applied	Number of experimented persons	Skinreaction after irradiation esp. pigmentation			Relation with the experiment of Urbach
		C>T	C=T	C<T	
80% bergamotoil alcohol	50	33	17	0	Coincides
50% glycerin alcohol	12	0	2	10	Not coincides
2% chrysarobin chloroform	10	0	2	8	"
Coal tar	10	0	0	10	"

C Controlled area
T Tested (applied) area

* By Minor Itô and Atsushi Nakajo. Published in Jap. J. of Dermat., 1942, 52, 132 (Japanese).

Table XV showed the results of the experiments in which vitamin C was substituted for other vitamins, and the results of the injection experiments of vitamin B_2 and vitamin B_2 plus C were the same as those of vitamin C.

TABLE XV

Pigmentation after the Application of 80% Bergamot oil Alcohol and Ultraviolet Irradiation with Another Vitamins or Hormones instead of Vitamin C

Substances subcutaneously injected	Number of tested persons	Skin reaction after irradiation esp. pigmentation			Relation with the experiment of Urbach
		C>T	C=T	C<T	
Vitamin B complex	15	0	0	15	Not coincides
Vitamin B_2	12	10	2	0	Coincides
Vitamin B_2+C	6	6	0	0	Coincides
0.1% adrenalin hydrochloride	15	12	1	2	Coincides
Adrenalcorticalhormone	12	3	2	7	Not coincides
Thyroidhormone	5	0	0	5	Not coincides

Experiment with hormones instead of vitamin C: As showed in Table XV only in the experiment in which ultraviolet rays were irradiated five or ten minutes after the injection of adrenalin the pigment production was inhibited and this is identical to the experiment of Urbach.

In regard to the mechanism of the experiment, Urabch assumed that the skin comes to be photosensitive by repeated applications of bergamot oil resulting in increased pigmentation after ultraviolet irradiation, but that the decomposition product of vitamin C combines with bergamot oil and inhibits its action on the skin. However, our experimental results with vitamin B_2 or adrenalin don't support this theory. As the specific chemical affinity of vitamin C to bergamot oil can't be admitted, the physical properties of this substance were examined in the following three experiments. 1) The refractory index of bergamot oil measured by Pulfrich's refractometer is not significantly different from other plant oils (tsubaki, olive, peanut, sesame, hydnocarpus, bellandonnaseed, rice, soyabean, castor and linseed). 2) The layer of bergamot oil does not coverge ultraviolet ray. 3) The ultraviolet absorption band of vitamin C together with bergamot oil is quite same to that of 80% bergamotoil-alcohol. Thus, the authors learned the mechanism of Urbach's experiment was due neither to the mere chemical antagonism nor to the physical characteristics of bergamot oil, so we made biochemical observation by the following animal experiment.

Animal experiment: Clipping the hairs of costoventral region of albino rabbit as shortly as possible, making nearly the same experiment as those of human body.

Results: The state of dermatitis was seen after applying bergamot oil or coal tar, and it became still more remarkable by additional ultraviolet irradiation. The inhibition of the inflammation through vitamin C or B_2 injection, was recognized neither macroscopically nor histologically; but the material being the albino rabbit, melanin could not, of course, be observed. When the penetration grade of topically applied

oil was tested by Sudan III in case of bergamot oil application, many lipoid granules were recognized at hair-follicle, its surroundings and all over the corium, (Plate XV, Figure 63) and the accumulation of lipoid was increased by the ultraviolet irradiation. But when the ultraviolet irradiation was performed together with vitamin C injection, that is, in case of the experiment same as one of Urbach, lipoid granules gathered at the surface of stratum corneum in thick layer, its penetration into epidermis was almost suppressed. In corium it was limited only around hair-follicle (Plate XV, Figure 64), and had not a bit of difference from nontreated control specimens (Plate XV, Figure 62). Nearly same results were obtained in the experiments in which oleum lavandulae, terebinthine and coal tar were used. By the way the results were the same when vitamin B_2 was used instead of vitamin C.

Histochemical examination of oxygen and reduction area by Unna's method:[2] The piece of skin used in the experiment on rabbit was examined and very interesting results were obtained; the reduction area was found at stratum corneum, hair-follicle and non-striated muscular fibers, and the oxygen area scattered in epidermis and cutis, besides around hair-follicle in the skin of non-treated normal rabbit (Plate XVI, Figure 65). When ultraviolet ray was irradiated, the oxygen area did not vary but reduction area around hair-follicle became more or less considerable. Besides there were no significant differences from the observation of non-treated rabbit to which the ultraviolet ray was irradiated together with vitamin C injection. But the specimen applying bergamot oil no notable alteration was found in the reduction area in comparison with the skin of non-treated rabbit. However peculiar alteration was found in oxyge area, *i.e.* it became remarkable in the lower part of epidermis and in the upper part of cutis (Plate XVI, Figure 66). The degree was still higher in the ultraviolet irradiated specimen. According to Urbach in the case to which vitamin C was injected, bergamot oil was applied, and ultraviolet ray was irradiated oxygen area became indistinct and on the contrary reduction area became distinct around hair-follicle and all over the epidermis and cutis (Plate XVI, Figure 67). The same alteration was proved when vitamin C was substituted for vitamin B_2.

In the case to which only coal tar was applied, no alteration was found in reduction area but oxygen area became considerable and its degree became higher by ultraviolet irradiation. This result is contrary to that of bergamot oil application.

Comment

From the above animal experiments the authors obtained two important findings. The first is the phenomenon that in the specimen stained by Sudan III, it was admitted, each application penetrated deeply into skin when it was used either alone or together with ultraviolet irradiation, but the penetrability of these oils into epidermis was inhibited remarkably by the vitamin C or B_2 injection. We have seen no report that the vitamin C or B_2 has the activity to decrease the permeability of the skin. But it is noticed that these vitamins contain negative group in their chemical constitution, therefore this evidence is compared with the fact that the substances which contained negative group have the inclination to diminish the

permeability of cell. That in the experiment of adrenalin injection the results were accordant with those of Urbach only when the interval of injection and ultraviolet irradiation was short, may be due to the immediate disappearance of the activity of adrenalin in short time.

The second is that in the experiment of vitamin C injection the considerable differences between bergamot oil and coal tar, which have the activity to increase the melanin production, were found in the investigation of oxygen area and reduction area by Unna's method. The chemical natures of both being considered that the bergamot oil is apt to be oxidized in air, thus at the surface of epidermis bergamot oil which has checked to penetrate into epidermis by vitamin C is oxidized, and acts reductively, so melanin production is inhibited, while coal tar is hard to change even when it is set in air, so it would display different results from bergamot oil.

Summary

The authors obtained quite similar results to those of Urbach's experiments, in which the inhibition of melanin production was recognized after the application of bergamot oil and ultraviolet irradiation following vitamin C injection. Furthermore the injection of vitamin B_2 or adrenalin instead of vitamin C showed almost similar results.

From the histochemical observations of rabbit by Sudan III and by Unna's oxidoreduction method, we confirmed that vitamin C or B_2 injection inhibits the penetration of topically applied bergamot oil. Thus it is guessed that bergamot oil is oxidized in air and supresses the melanin production as a reducing agent.

References

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XVII. Relationship between Melanin Production and Dioxybenzenes*

Oettel[1] recognized in his experiment of hydroquinone intoxication that in chronic intoxication the black cat are decolorized and turned in gray-white and guessed it being due to the interruption in the process of oxido-reduction. Getting the suggestion from his experiment, we made the experiment on goldfish, using hydroquinone and its isomers, resorcinol and pyrocatechol.

Method: The black goldfishes (*Kurodemekin*—Plate XVI, Figure 68) used in this experiment are black since the brood and their body color does not vary for life and the black pigment is believed to be melanin. Goldfishes weighing 20–40 g. were fed in water temperature at 15°–20° C. 0.5% saline solutions of resorcinol, hydroquinone and pyrocatechol respectively were made and 0.05 cc. of each solution was injected subcutaneously every other day in the ventral region of *Kurodemekin*.

Results: The results are shown in Table XVI.

TABLE XVI

Decoloration following Dioxybenzenes Injection on Black Goldfish

(The sign ### shows the original black tone of *Kurodemekin*, ###, ##, +, − shows the degree of black tone in turn)

	Number of fishes	Before injection	After		
			5th inj.	10th inj.	15th inj.
0.9% NaCl 0.05 cc.	5	(###)	3 (###) 2 d*	3 (###)	3 (###)
0.5% resorcinol 0.05 cc.	5	(###)	4 (###) 1 d	3 (###) 1 d	3 (###)
0.5 hydroquinone 0.05 cc.	5	(###)	4 (##) 1 d	2 (+) 1 (−) 1 d	2 (−) 1 d
0.5% pyrocatechol 0.05 cc.	5	(###)	2 (###) 3 d	2 (###)	2 (###)

* Number of dead fishes during the experiment

In hydroquinone the fading began at the third injection, the red-yellow tone extended gradually from the injected part to costo-abdominal region, tail and head equally (Plate XVI, Figure 69), and after tenth injection the goldfish became indistinguishable from red one (*Akademekin*—Plate XVI, Figure 70). The margin of fins, bulbi and palpebra, however, remained black, and the decoloration of the

* By Minor Itô and Atsushi Nakajo. Published in Jap. J. of Dermat., 1942, 51, 295 (Japanese).

pigment was not recognized. The injections of resorcinol, pyrocatechol and saline (control) show no decoloration at all.

The goldfish decolorized by hydroquinone injection regenerated the black pigment slowly and diffusely from the margin of fins where the black pigment remained in about three weeks after the end of the injection, and became the original *Kurodemekin* in forty days (Table XVII).

TABLE XVII

Number of fishes	After the last injection				
	18 days	30 days	42 days	45 days	50 days
2(−)	1(±) 1(−)	1(+) 1(+)	1(++) 1(++)	1(++) 1(++)	2(++)

Influence of sunlight and temperature of water: The black pigment of amphibia or fish, etc. is often influenced by sunlight or temperature. We performed the experiments, changing these conditions. In regard to the conditions of sunlight we made three gradations of intensity of illumination; the first was " dark," in which case the aquarium of goldfish was covered with black cardboard, the second is "lighted" in case of which no direct rays were shone, and the last is "well lighted" in which the aquarium was set at the side of window towards the south. In consequence of the experiments of hydroquinone injection to five goldfishes in each conditions, the decoloration appeared in "well lighted", in "lighted" and in "dark" in turn and the regeneration of the black pigment after the last injection was fastest in "well lighted" revived completely in month, and was latest in "dark."

In respect of the water-temperature, making the experiments in greenhouse in winter under the following conditions; temperature below 15° C, 15°–20° C, and above 20° C. We found both depigmentation and pigmentation was fastest in case of the optimal temperature (15°–20° C) to goldfish.

Experiment with vitamins, hormones, etc.: vitamin C, B_2, B-complex, adrenocortical hormone, glutathione, albalite (bleaching agent), etc. were injected on black goldfish by the same method to that of hydroquinone but no depigmentation was observed. Merely the light brownish red color was observed after 20 minutes of 0.05 cc. adrenalin injection (0.1%) but it returned to black after several hours.

Histological investigation of skin and scale of goldfish: Large quantity of melanin were contained in the scales and epidermis of black goldfish, and yellow-scarlet lipochrome mixed irregularly with them. Melanin granules of scales and epidermis decreased by the hydroquinone injection, melanin disappeared completely in the specimen which was excised after seventh injection, and dopa reaction turned to negative. But the chromatophores in corium were kept normal throughout the experiment and were not influenced by hydroquinone. Microscopically the temporaly fading of melanin following the adrenalin injection was the contraction of melanin granule and the dopa reaction was always positive at the same degree.

COMMENT

It seems reasonable to consider that melanin is decolorized by reducing potency of hydroquinone as Oettel guessed, but we can't be convinced of it because the decoloration of melanin was not recognized in case of pyrocatechol which has stronger reducing potency. If the injection of hydroquinone induces regressive metamorphosis in epidermis, it is not explained why it acts only on epidermal melanoblast selectively, and causes no alteration in other tissues.

In order to make clear the character of hydroquinone, the alkalization potency of skin was examined by the litmus cutaneous test, according to Leszczynski and Falik's[2] method.

Method: The alkalization potency is indicated as follows; red litmus solution (*p*H 6.46) and blue litmus solution (*p*H 7.0) are injected intracutaneously at the same time, and the duration that it took till both red and blue wheals change into the equal dark blue is measured.

In this experiment albino rabbits were used instead of goldfish, and litmus cutaneous test was made after half an hour after making previous subcutaneous injection of the dosage of one hundred times as much than goldfish culculating from dosis letalis minima of dioxybenzenes to godfish and rabbit. The results were shown in the Table XVIII.

TABLE XVIII

Alkalization Potency measured by Leszcynski-Falik's Litmus Method

	Animal number	Neutralization time				
		Before injection	After		After the last injection	
			1st inj.	3rd inj.	1 day	7 days
2% hydroquinone 1.25 cc.	1	20′ 40″	36′00″	43′20″	38′00″	36′45″
	2	21 05	37 40	44 40	40 32	38 15
	3	20 18	37 20	46 30	38 15	37 00
2% pyrocatechol 1.25 cc.	4	20 50	54 15	56 00	25 12	20 18
	5	22 20	56 05	54 00	22 16	19 30
	6	19 00	55 00	55 18	14 18	21 00
2% resorcinol 1.25 cc.	7	19 45	22 33	20 30	18 40	20 15
	8	20 35	23 15	18 10	22 15	22 00
	9	22 18	21 46	19 45	20 18	22 30
2% vitamin C 1.25 cc.	10	18 45	21 30	15 20	17 00	20 15
	11	20 18	20 45	14 40	18 30	21 00
	12	21 32	20 45	14 30	18 50	22 00

While the time of normal litmus test in rabbit skin was 19–22 minutes, it lengthened twice upwards at the first or third injection of hydroquinone and pyrocatechol. The lengthened values by hydroquinone showed for a week after the last injection, but by pyrocatechol returned next day to the normal value. It is learned from the results that resorcinol is stable, excludes out of body without alteration in vivo and pyrocatechol is labile, changes easily, and stands still for short time in vivo. Contrary to them, as the degree of stability of hydroquinone is moderate and it stays long in body, it is guessed that the reducing potency of it is desplayed thoroughly,

and the alkalization potency of the injected body, it is appeared, is suppressed, consequently the melanin production which is the oxidation is diminished. While dopa reaction was weakened in the section fixed with acid formalin, but (on the contrary) it can be proved after several days in the sections fixed with neutral one, and this fact supports our inference. That is, it is guessed that the hydroquinone injection causes the disorder of alkalization potency of skin and lets the dopa-oxydase reaction weaken, and at last suppresses the melanin production.

Summary

Fading of melanin in the epidermis and scales was found by hydroquinone injection on black goldfish. Dopa reaction turned to negative after the decoloration had been completed, but the melanin in the chromatophore remained unchanged. Optimal temperature for this process was 15°—20° C., and ultraviolet ray accelerated both depigmentation and repigmentation.

Two isomers of hydroquinone, resorcinol and pyrocatechol, however, were proved ineffective in spite of the stronger reducing potency of the latter than hydroquinone.

The mechanism of the depigmentation by hydroquinone could not be explained by mere reduction of melanin, but rather by the special suppression against dopa oxydase through its alkalization potency.

The temporaly fading by adrenalin injection was microscopically the contraction of melanin granules.

References

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XVIII. Influence of Benzoquinone on Melanin Production*

The experiments of melanin production, using benzoquinone which is the catalyser in the metabolism of amino acid and is chemically contrary at all to hydroquinone which has the decolorizing action of melanin mentioned in the previous report were performed.

Method: The yellow-red and white goldfish (*Wakin*) were used. The condition of the feeding and the method of injection were the same as the previous report; 0.5% saline solution of *p*-benzoquinone was freshly made, and 0.05 cc. of it was injected subcutaneously every other day at the costoventral region of goldfish.

Results: The results obtained were shown in the Tables XIX and XX.

TABLE XIX

Black Pigment Production following *p*-Benzoquinone Injection on Yellow-Red Goldfish

(−, ±, +,.....卌 Grade of the pigmentation)

	Number of fishes	Before injection	After			
			3rd inj.	5th inj.	8th inj.	10th inj.
0.9% NaCl 0.05 cc	5	(−)	5 (−)	4 (−) 1 d*	3 (−) 1 d	3 (−)
0.5% *p*-benzoquinone 0.05 cc.	10	(−)	3 (±) 5 (−) 2 d	2 (+) 5 (+) 1 d	2 (卌) 4 (+) 1 (+)	6 (卌) 1 (+)

* Number of dead fishes during the experiment

TABLE XX

Fading of the Black Pigment after the Last Injection of *p*-Benzoquinone

Number of fishes	After the end of injection				
	15 days	30 days	50 days	75 days	95 days
7 (卌)	2 (卌) 5 (卌)	3 (卌) 4 (卌)	5 (卌) 2 (+)	4 (卌) 1 (+) 2 d	1 (卌) 3 (+) 1 (−)

As the influence of *p*-benzoquinone injection to the yellow-red goldfish (Plate XVI, Figure 71), black-brown pigment production was seen at the injected part or the margin of fins after the third injection, the black tone strengthened and extended. As the number of injection goes on the godfish showed thorough the appearance of

* By Minor Itô and Atsushi Nakajo. Published in Jap. J. of Dermat., 1942, 52, 1 (Japanese).

black one after the tenth injection (Plate XVII, Figure 72), *i.e.*, after twenty days from the beginning of the experiment. The strength of the black tone could be recognized still for about one month after the end of injection (Plate XVII, Figure 73), but the fading of the black color was seen gradually in two or three months after the end of injection but it took considerable time to return to the original yellow-red one.

The results of the experiments made on yellow-red goldfish and white one with or without yellow-red spots were shown in the Table XXI.

TABLE XXI

Black Pigment Production following *p*-Benzoquinone Injection on White-Yellow-Red Goldfish and White Goldfish

	Number of fishes	Before injection	After			
			5th inj.	10th inj.	13th inj.	15th inj.
Yellow-red goldfish	3	(−)	1 (++) 1 (+) 1 d	1 (++) 1 (++)	2 (++)	2 (++)
White goldfish with yellow-red spots	3	(−)	1 (+) 1 (±) 1 d	1 (++) 1 (±)	1 (++) 1 (±)	1 (++) 1 (+)
White goldfish	6	(−)	3 (−) 3 d	1 (±) 2 (−)	2 (++) 1 (+)	2 (++) 1 (+)

The white goldfish was high in the rate of death, the black pigment production was very slow, and the injected part and the margin of fins became black at first after the fifteenth injection. In white goldfish with yellow-red spots (Plate XVII, Figure 74) the considerable black color tone appeared in the yellow-red spots and at the margin of fins after about 12th injection (Plate XVII, Figure 75).

Influence of sunlight and temperature of water: In consequence of the experiment in yellow-red goldfish under the same conditions to the previous report, the black color tone appeared earliest in "well lighted" and latest in case of "dark." In regard to the water temperature most considerable black tone appeared at the optimal temperature (15°—20° C). By the way, it is very interesting that two white goldfishes with yellow-red spots among those in which the black tone was produced by benzoquinone had faded once after the experiment, and remained as a white yellow-red one during the winter, but the said black spots as were seen in the previous experiment reappeared again unexpectedly at the middle of May next year, and this may be, it is guessed, because the metabolism of goldfish was promoted by the influence of sunlight and the temperature.

Experiments with additional vitamins and gluthatione: It must be noticed that in white yellow-red goldfish the black pigment production due to the previous injection of *p*-benzoquinone was remarkable in the region in which lipochrome situated. Lipochrome is a compound of intricate composition, and its principal component is carotinoid pigment. This reminds us of the relation between carotinoid and vitamin A, so we used this with *p*-benzoquinone. In the experiment, using vitamin C, B_2 and gluthatione, etc. with *p*-benzoquinone, the black pigment appeared more

remarkable as shown in Table XXII, in case of that vitamin A, C, B_2 and gluthatione were used accompany with *p*-benzoquinone than that only *p*-benzoquinone was injected.

TABLE XXII

Experiment adding Vitamins and Glutathione on Yellow-Red Goldfish

	Number of fishes	Before injection	After		
			5th inj.	10th inj.	14th inj.
1.0% *p*-quinone	5	(−)	2 (++) 2 (±)	1 (+++) 2 (++)	3 (+++)
1.0% *p*-quinone + vitamin A	5	(−)	2 (++) 2 (+)	3 (+++)	2 (+++) 1 (+++)
1.0 *p*-quinone + vitamin B_2	5	(−)	2 (+) 1 (±)	2 (+++) 1 (++)	1 (+++) 2 (+++)
1.0% *p*-quinone + vitamin C	5	(−)	1 (++) 4 (±)	4 (+++)	1 (+++) 3 (+++)
1.0% *p*-quinone + glutathione	5	(−)	2 (++) 3 (+)	2 (+++) 2 (++)	1 (+++) 2 (+++)

Histological investigation (*Plate XVIII, Figures* 77–82)*:* There were no melanin in the scales and epidermis of yellow-red goldfish, there was only yellow-red lipochrome, and dopa reaction was negative. In case of white goldfish there was no pigment cells, but mere guanine crystal. Histological investigation of the scales and skin of the yellow-red goldfish which became visible black following the *p*-benzoquinone injection, many black-brown pigment granules were produced newly in the scales and epidermis, mixed with the ready-existing lipochrome which became almost invisible as the degree of black color tone became distinct and the same appearance as the microscopic observation of the scales and epidermis in the black goldfish (*Kurodemekin*) is observed. Dopa reaction turned obviously to positive and the black pigment faded after several hours' immersing in hydrogen peroxide.

Chemical investigation of newly produced black pigment: The nature of melanin being yet not made clear, its identification is very difficult, and there are only the following standard of it; dopa reaction, the silver reducing agency, the decoloration by hydrogen peroxide, and the hard solubility in most solvents, etc. but all these are not always precise. Therefore, it must be disputable to consider the black pigment produced by benzoquinone as melanin. We tried with many kind of solvents in order to examine by spectroscopical method, but could not succeed to dissolve it at all. Only light-brown extract was obtained by the method of Przibram[1] and Tutshku;[2] it is to immerse the tissue in the solution consists from 9 parts of 75% alcohol and 1 part of ether and set at the dark, cool place for ten weeks. Then the extract is treated with sulphuric and nitric acid till the lipochrome reaction turns negative. The black human hair, the skin-piece of nevus pigmentosus and the sepia of cuttlefish were compared with spectroscopically as the control by the same procedure. The contineous absorption band was shown, at the center of which was 4000 Å, and the special absorption spectrum was not seen both in the extract from scales or skin of goldfish and those of controlled materials. This result was the

same as was reported by Danneel,[3] and it is guessed that it is owing to the complex constitution of melanin. Thus I guessed newly produced black pigment is melanin from these chemical natures.

Experiments with tyrosine, dopa, pyrrol and adrenalin: We made the experiments in yellow-red goldfish, using tyrosine, dopa, pyrrol, tryptophan and adrenalin, those which are considered as melanogen; hydrogen peroxide as the oxidizing agent, copper or gold preparations as the catalyser, and pilocarpine as the contrast to adrenalin with the single injection or combined with the ultraviolet irradiation, but in any cases the black pigment was not produced.

Comment

In regard to pigment experiment by benzoquinone, only Mayer's[4] experiment in vitro is cited but it is not useful for our purdose. The fact that black-brown color tone appeared first at the margin of fins distant from the injected part coincides with the fact that the margin of fins remained black even when the fading of melanin was noticed in the hydroquinone experiment of the previous chapter. Therefore it is clear that the black substance appeared by *p*-benzoquione injection was not the deposit of exidized benzoquione. The periphery of fins is widest in the external surface of body and so the degree of absorption of oxygen from water is highest and oxydase is activated sufficiently. Moreover the fact that the red spots turned to black selectively in white yellow-red goldfish in our experiment can't be considered as the result of deposition of oxidized benzoquinone. In the spectroscopic test, *p*-benzoquinone which becomes black when left standing in air presented the obvious absorption band at 3600Å. This is quite different from the continuous absorption band of black-brown extracted solution obtained from the tissue after *p*-benzoquinone injection.

Thus from the abovementioned histological and chemisal observation (p. 94) we guessed that the black pigment produced in the experiment of *p*-benzoquione is melanin.

The chemical process of melanin production is the difficult problem, which has not been made clear, but the hypothesis of Evans and Raper explains it as follows; tyrosine turns to dopa by tyrosinase, it is oxidized by dopa oxydase to dopa-quinone, and afterwards becomes black-brown melanin after oxidizing without requiring enzyme. In view of this hypothesis, the results of our experiment are explained as follows. No dopa reaction was found both in epidermis and scales of yellow-red and white goldfish but the injection of *p*-benzoquinone dopa reaction turned to positive and melanin production was observed. Thus *p*-benzoquinone is considered as a biocatalyser. This was confirmed by the observation that melanin production increased when such activator like vitamin C, B_2 or gluthatione was added. In regard to the relationship between melanin and lipochrome, there are the treatises of Kutschera-Aichbergen[5] and Abeloos[6], etc. The close correlation between melanin production and carotinoid substance is surmised according to the following facts obtained from our experiment; the first is that the melanin is found mixed with lipochrome in black goldfish and the second is that vitamin A which was considered as a biocatalyser by Euler and Hellström (1928) presents the activator-action.

SUMMARY

The black pigment was found after *p*-benzoquinone injection in yellow-red or white goldfish and dopa reaction turned to positive at the same time.

The pigmentation took place earlier in goldfish with pre-existent lipochrome granules than in white one.

Temperature and sunlight were found to influence the melanin production.

This pigment production was accelerated by injection of vitamin A, B_2, C or gluthatione.

The black extract of the newly pigmented scales and skin of goldfish presented all chemical and physical characteristics of human melanin.

Thus it is guessed that *p*-benzoquinone participated in the melanin production as a kind of activator.

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XIX. Influence of Hormones and Others on Melanin Production*

In lower vertebrates their color change is due to the concentration or dispersion of melanin exists in pigment cells. These pigment cells concerned are under the control of the sympathicus, and so it is generally recognized that the endocrine influence which is closely connected with the sympathicus has a great importance. In higher vertebrates the direct relation between the pigment cell and the nervous system cannot be elucidated, but Jores[1] observed that the human melanophore-diffusing hormone is related to the dark adaptation which causes the movement of retinal pigment. In addition to that, the correlation between the pigment of skin and the autonomic nerve or the endocrine status in the human body is able to know in Addison's disease, menstruation and gravidity, but the most part of this question cannot be elucidated as yet.

We have investigated in order to make up previous experiment[2] and observed the color change of goldfish caused by various hormones and poisons of vegetative nervous system.

Experiment on Black Goldfish (*Kurodemekin*)

Materials and methods of this experiment are quite same as Itô and Nakajo's.[2]

Results: It is generally recognized that the concentration of melanophore in fishes is provoked by feeding with epinephral substance or by injection of adrenalin, and Itô–Nakajo's experiment confirmed these facts. We observed in the same way, the temporary decoloration due to the melanophore concentration which is provoked by injection of ephedrin or wake-amines (1-phenyl-2-methyl aminopropane hydrochloride), both of them have sympathicotropic action. On this experiment, the decoloration provoked by injection of ephedrin was less intense than that of adrenalin, but was much more durable; when provoked by wake-amines, the decoloration was slight and continued only short time. Althouth the melanophore concentrating action with ergotoxin is observed by Barbour and Spaeth[3] on fishes, by Leszczynski[4] on frog, but this action is very slight in our experiment. (Table XXIII)

It has been known that chinine or cocaine is capable of giving positive melanophore reaction, but when adrenalin was taken with them, the melanophore concentrating action of adrenalin was suppressed slightly.

It is confirmed by many investigators that the injection of insulin in large dose presents the increased adrenalin in blood as the results of hypoglycemia. Therefore we attempted the experiment by the injection of insulin in large dose but no decorolation in the skin of goldfish was observed. Injection of vitamin P had no influence upon this experiment.

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* By Minor Itô and Masahiko Watanabe. Published in Tohoku Igaku Z., 1947, **36**, 1 (Japanese).

TABLE XXIII

Temporary Decoloration on Black Goldfish

(The sign ⫼ shows the original black tone of goldfish, ⫼, ⧺ and + show the degree of black tone in turn)

	Number of fishes	Before injection	After injection						
			10′	30′	1 h	3 h	5 h	24h	48h
0.1% adrenalin 0.05 cc.	5	⫼	⧺	±	±	⧺	⫼	⫼	⫼
4% ephedrin 0.05 cc.	2	⫼	+	+	+	+	⧺	⧺	⫼
Wake-amine 0.05 cc.	1	⫼	⧺	⧺	⫼	⫼	⫼	⫼	⫼
0.1% ergotoxin 0.1 cc.	3	⫼	⫼	⫼	⫼	⫼	⫼	⫼	⫼
0.1% adrenalin 0.1% chinine āā 0.05 cc.	2	⫼	⧺	⫼	⫼	⫼	⫼	⫼	⫼
0.1% adrenalin 0.5% cocaine āā 0.05 cc.	2	⫼	⧺	⫼	⫼	⫼	⫼	⫼	⫼

Experiment on Yellow-Red Goldfish

Though it is generally recognized that the specific action of pituitary hormone disperses, melanophore, yet chinine, cocaine, acetylcholine, caffeine, yohimbine, strychinine, acetic acid, etc. are also the substances which give the positive melanophore reaction although they have no specificity. In addition to that, Zondek and Krohn[5] found in their experiment on *Phoxinus laevis* that the matrimony lobe is due to the dispersion of black and red pigment and is selectively formed by the administration of intermedin. In Komori's[6] experiment on *Acheilognathus tabira*, the matrimony lobe was selectively formed by pituitary hormone, and it was also formed by yohimbine, morphine, ergotine, chloral hydrate, strychinine, ether, chloroform and atropine. Lipshutz, Bloch and Schrafl,[7] Tsubota[8] observed hypertrophy of mamma and hyperpigmentation of mammilla by the application of ovarial hormone in their experiment on guinea-pig.

As indicated on Table XXIV in the series of our experiments with hormones and poisons, we could observe the production of black pigment by injection of pituitary hormone of the anterior lobe, acetic acid and of hematoporphyrin. The gonadotropic hormone extracted from urin could not produce the black pigment, but by applying pituitary hormone of the anterior lobe (prolan A), we have observed the positive result coincides with the process of the melanophore reaction, and from this fact, we suppose that this hormone plays probably the part of Euler's socalled hormozyme. It was described that the injection of diluted acetic acid—Thumberg called it a sort of metabolic substance—has a melanophore dispersing action. In our experiment also, we observed the production and the enlargement of black fleck. Hematoporphyrine is a substance with photodynamic action; applying it in our experiment at the same time with the ultraviolet irradiation, we observed the increased

TABLE XXIV

Pigment Production with Hormones or Poisons on Yellow-Red Goldfish

(−, ±, +, ++.....Grade of the pigmentation)

	Number of fishes	Before injection	After			
			5th inj.	10th inj.	15th inj.	20th inj.
Pituitary hormone (anterior lobe)	3	−	1 (++) 1 (±) 1 (−)	1 (+++) 1 (+) 1 d*	1 (+) 1 d	
Gonadotropic hormone from urin	6	−	−	−	−	−
Pituitary hormone (posterior lobe)	4	−	−	−	−	−
Thyroid hormone	2	−	1 (−) 1 d	1 d		
Testosterone	2	−	−	−	−	−
Luteosterone	2	−	−	−	−	−
Folliculin	2	−	−	2 (±)	2 (±)	2 (±)
0.8% acetic acid	2	−	−	2 (+)	1 (+) 1 d	1 d
Yohimbine	2	−	−	1 (−) 1 d	1 (−)	1 (−)
Hematoporphyrin	3	−	−	1 (+) 2 (−)	1 (++) 1 (−) 1 d	1 (++) 1 (−)
Hematoporphyrin with ultraviolet ray	2	−	1 (+) 1 (−)	1 (++) 1 (+)	1 (+++) 1 d	
Acetylcholine	4	−	2 (−) 2 d	2 (−)	2 d	

* Number of dead fishes during the experiment

melanin production in the region about the dorsal fin of goldfish (Plate XVII, Figure 76).

SUMMARY

Adrenalin, ephedrin, such wake-amines as 1-phenyl-2-methyl aminopropane hydrochloride could produce temporary decoloration in black gold-fish. Total and permanent fading of melanin, however, could not be achieved with these substances.

Pituitary hormone of the anterior lobe, hematoporphyrin and acetic acid produced black pigment in yellow-red goldfish. Other substances examined (thyroid hormone, pituitary hormone of the posterior lobe, acetylcholine, etc.) were found useless.

From these facts it is clear that complete decoloration of melanin is difficult by injection of melanophore-dispersing substances, and a special bio-catalytic substance such as hydroquinone is required for this purpose.

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XX. General Conclusion*

In summarizing our experiments mentioned in the foregoing chapters, the following conclusions can be reasonably reached.

The Participation of Nervous System in Melanin Production

By the results of Bielschowsky-Seto's silver impregnation method, it is found that nevus cell of pigmented nevus originated from neuroectodermal system which is homogenetical to Schwannian cells just as Masson stated. In an autopsy case of abortive Recklinghausen's disease without neurofibroma, preneurinomatous changes were noticed microscopically in the pigmented area and in solar plexus. Further circumscribed increase of Schwannian nuclei of larger nerve bundle is observed in the deeper dermal layer of pigmented area. These findings disclose the fact that there exists close relationship between Recklinghausen's fleck and neurofibroma, and are influential data of nerve cell theory stated by Verocay, Held, Kyrle, Masson, Cailliau, Jausion-Levy-Coblentz-Spillmann and recently by John and Ormea.[1] The frequent occurrence of socalled neurofibromatous structure of nevus cell is reported by Becker[2] who in the histological study of 741 cases of pigmented nevi found in 72.5% the structure of the third type of Miescher.[3] Thus the nevus cell which may present polyomorphism according to the local peculiarities, is uniformly of neuroectodermal origin. These facts give clue also to the polymorphic histological picture of melanocarcinoma.

On the contrary, the pigment cell of Mongolian spot, blue nevus and nevus fusco-caeruleus belong to mesenchymal melanoblast. In the latter two diseases which are properly interpreted as localized malformation, the histological structure is apparently more exaggerated than in physiological character *i.e.* Mongolian spot, and the affinity to nervous system is more conspicuous suggesting the coincidence with endo- or perineurium. This finding as well as the results of phylogenetical investigation of Mongolian spot, leads us to the conclusion that the pigment cell in these dermatoses belong to homogenetical melanoblasts in the sense of perineurale Pigmenthülle of Weidenreich. Though Mongolian spot disappears ontogenetically in a prescribed duration in which it becomes estranged itself from nervous system and on the contrary, blue nevus and nevus fusco-caeruleus are interpreted as the localized malformation close to nervous system and exist for life. This reminds one of the importance of correlation between melanin formation and nervous system. Thus we were able to verify both histologically and genetically that both ectodermal and mesodermal melanoblasts are closely related to nervous system. The conception of mesectoderm of Platt[4] which was introduced into the genetical experiment on the mechanism of development of pigment cell and pigment flecks in amphibia by Harrison,[5] Holtfreter, Du Shane,[6] Twitty,[7] Rosin, De Lanney and Tada is of interest in this connection.

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* By Minor Itô.

In many clinical cases of systematized nevi, incontinentia pigmenti, nevus ophthalmo-maxillaris Ota and Mongolian spot, the eruption are localized in such a manner as one can easily assume the coincidence with the distribution of cutaneous nerve. Such cases are reported in the corresponding chapters. In various pigment disorders, functional abnormalities of vegetative nervous system are disclosed by the results of histamine scratch test and diaphoretic test. In some cases of pigmented nevus and incontinentia pigmenti, the abnormality of trophic nerve distribution resulted in the asymmetry of the breast glands. In several cases of nevus spilus en nappe, even the sensible nerve was involved in the affection. These are homolateral nervous disturvances accompanying pigmentary disorders and are favorable to our theory.

In benign malanomas, I believe, it is possible to distinguish rather distinctly those originating from Schwannian cellular ectodermal melanoblasts and those from mesenchymal melanoblasts corresponding to endo- or perineurium. So it is theoretically not impossible to differentiate histologically melanosarcoma and melanocarcinoma. This differentiation, however, is not always possible in individual cases. We believe that this problem will be given a solution in further progress in the investigation.

The Rôle of Enzymes in Melanin Production

The conflict of the monism and dualism on this problem was already mentioned. We tried indophenolase reaction in the melanoblasts which were considered as dopa positive by many investigators such as cells of basal layer, pigmented nevus, Mongolian spot, blue nevus, nevus fusco-caeruleus and malignant melanoma or pigment cell of Riehl's melanosis and found numerous indophenolblue granules in their protoplasm. Their size and form coincide quite well with those of preexistent melanin granules. And they are more numerous in cells with little melanin content. While on the other hand positive peroxydase reaction is principally limited to nuclei, and the reaction is especially strongly positive in the nuclei of basal cells, nevus cells and dermal melanoblasts, where diffuse or granular staining of protoplasm is also noticed. Endothel cells of blood capillaries present strong positive reactions, and indophenolblue granules and peroxydase positive granules overflow to the surrounding tissue. And at the same time local blood leucocyte of pigmented area shows stronger peroxydase reaction than normal area. This shows that there occurs exchange of both enzymes between myeloic leucocytes and tissue cells. These biochemical reactions exhibit the passing phase of melanin production in the presence of two enzymes, oxydase and catalase in the protoplasm. In permanganate methylgreen staining of Unna the site of melanogenesis stains always with methylgreen as oxygen areas. And in vitiligo the papillary layer reduces permanganate as reduction areas. In melanoma amelanotic foci also reduce permanganate, like nevus cell nest without melanin. Thus the lack of melanogenesis in malignant melanoma is no less than a phase of extremely rapidly metastatic and infiltrative growth of tumor cells and cannot be estimated as a measure of malignancy. This finding coincides with the experimental results of Greenstein[8] on the oxygen consumption of melanotic and amelanotic mouse melanoma suspension.

It is well known that malignant melanoma is rare in Negros and that it frequent-

ly occurs in area which is physiologically poor in pigment such as finger tip, sole and lip. So it may be properly assumed that the disturbance of biochemical condition resulting in abnormal melanin production in heterotopic area is a cause of malignancy like the incidence of malignant melanoma in white horse.

In our follow-up study of Urbach-Kral's[9] experiment, the increase of oxygen area is noticed in area hyperpigmented by the application of bergamot oil. The injection of ascorbic acid and riboflavin prior to the application of bergamot oil and ultraviolet irradiation completely inhibits hyperpigmentation. On histochemical examination the increase of reduction area is noticed. By injection of these vitamins the cutaneous penetration of bergamot oil is reduced and the decomposition product of this substance reacts rather reductively on the skin resulting in the suppression of melanin production. In Riehl's melanosis the deficiency of vitamin C and B complex is surmized by the increased value of pyruvic acid and iodic acid reducing substance of blood serum. These vitamin deficiencies are important etiological factors in Riehl's melanosis. The abovementioned results of Unna's staining, indophenol oxydase reaction and peroxydase reaction point to the importance of local biochemical condition specially of redoxpotential and the action of biocatalyst in normal and pathological melanogenesis.

In the experiment with black goldfish, only one of the three isomers of dioxybenzenes, namely hydroquinone can suppress melanin production. This action is attributed to the decreased alkalization potency of cutaneous tissue by hydroquinone. Recent observation of Dejust, Ropshow, Cailliau[10] on the dermoepidermal hydrogen ion concentration supplies further influential data to this theory.

On the other hand, by injection of *p*-benzoquinone to yellow-red goldfish, black pigment is formed and the dopa reaction of scale turned to positive. This newly formed pigment is identified as melanin. As all other oxidizing agents failed to give similar results, it is not a mere oxidation by *p*-quinone but a special catalytic action of this substance on melanogenesis.

That the enzyme tyrosinase is indispensable for melanogenesis is admitted by almost all investigators.[11] This ferment is actually identified in the extract of human and animal melanosarcoma by Niklas.[12] In normal human tissue, however, it has been impossible to obtain tyrosinase activity. This may be due to the presence of inhibitor substance like ascorbic acid and sulfhydryl compounds (Hoff[13]). While on the other hand Bloch and Raper emphasized the participation of dopa oxydase. And in my experiment indophenolase and peroxydase are observed in the histochemical study, so their participation in melanogenesis is readily surmised. Thus I believe the two enzymes theory is more probable. Then how is the action of *p*-quinone in the goldfish experiment explained? According to Mann and Keilin[14] in the presence of small amount of *o*-quinone polyphenolase catalyzes not only polyphenole but also monophenole. *p*-Benzoquinone may act in the same way as an activator and thus accerelate melanogenesis. Both indophenoloxydase and peroxydase has common substrate and their difference lies only in acceptor substance. So their activities are presented at various passing phases of biochemical condition of individual tissue cell. The manifestation of dopa oxydase activity represents a phase of this cellular activity and cannot always be estimated as the existence of specific enzyme. This pigment production in goldfish appears first and most

prominently at the place where carotinoid pigment is preexistent. And the addition of vitamin A, B_2 and C to *p*-quinone accelerates melanin production. So the participation of cytochrom system cannot be overlooked.

From the above histological and histochemical researches, I have concluded that melanogenesis is a part of biological oxidizing processes which is carried on under the influence of nervous system. In this sense the chemical transmission theory of Loewi–Dale and/or neurohormonal theory of Vilter is introduced in the mechanism of melanogenesis.

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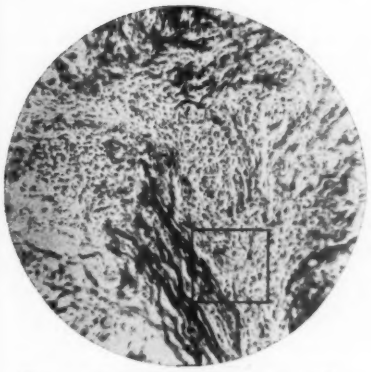
PLATES

Fig. 1.

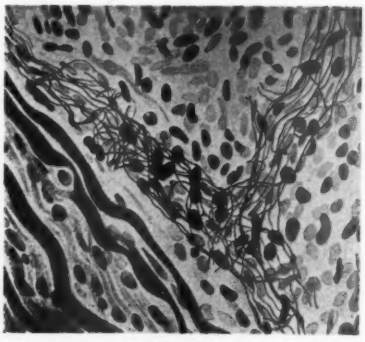


Nevus cell at the deeper layer of the cutis. (Neurofibril staining)

Fig. 2.

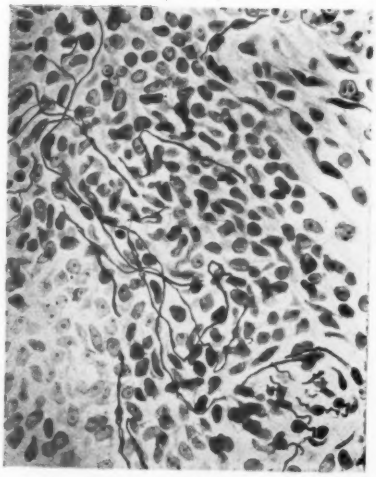
Nevus cell at the reticulay midlayer. (Neurofibril staining)

Fig. 3.



High power drawing of the marked part in Fig. 1., Schwannian nuclei giving the impression of transition to nevus cells.

Fig. 4.



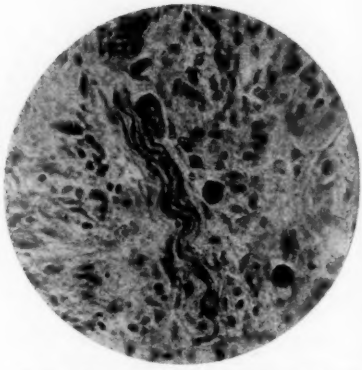
High power drawing of the marked part in Fig. 2., Neurofibrils running into the nevus cell nest.

PLATE II

Fig. 5.

Nevus cell nests contact with the course of neurofibrils and fine vegetative nerve.

Fig. 6.



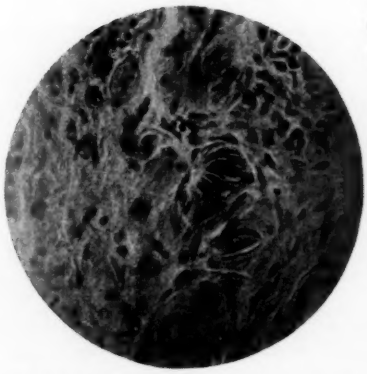
Isolated nevus cell contacts with the course of neurofibrils.

Fig. 7

A. Groups of nevus cell in subpapillary layer.

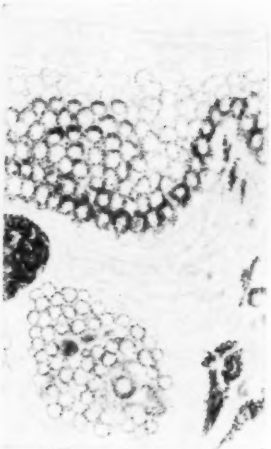
C. Neurofibromatous structure of nevus cell in reticulary midlayer.

Fig. 8.



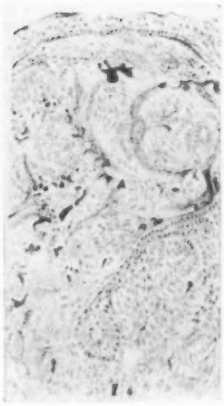
Isolated nevus cell contacts with the course of neurofibril and corpuscule naevique.

Fig. 9.



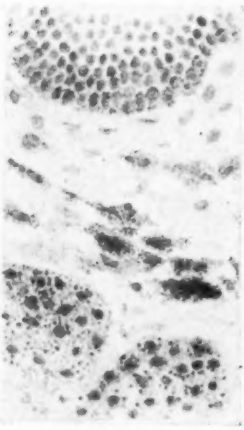
Nevus pigmentosus.
Labile oxydase reaction
Positive granule.....blue.
(Gräff's Nadi method)

Fig. 10.



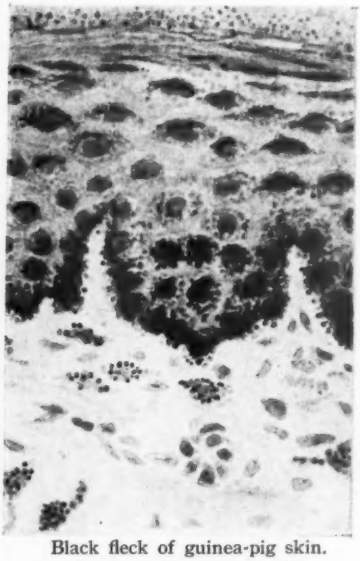
Nevus pigmentosus.
Peroxydase reaction
(Copper benzidine staining)

Fig. 11.



Nevus pigmentosus.
Labile peroxydase reaction
Positive granule.....blue.
(Okano's C method)

Fig. 12.



Black fleck of guinea-pig skin.
Labile peroxydase reaction
Positive granule.....blue.
(Okano's C method)

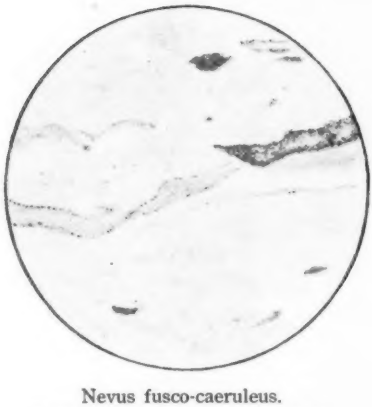
Fig. 13.

Nevus pigmentosus.
Oxygen reduction staining
(Unna's method)

Fig. 14.

Mongolian spot.
Peroxydase granule .....blue,
melanin granule .....brown.
(Okano's C method,
oil immersion)

Fig. 15.



Nevus fusco-caeruleus.
Dopa reaction in reticulary layer
Dopa positive granule.....black,
melanin granule .....brown.
(Oil immersion)

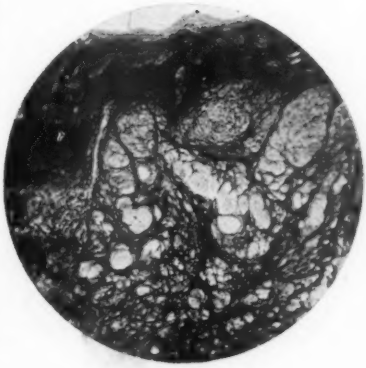
Fig. 16.

Nevus fusco-caeruleus.
Oxygen reduction staining
(Unna's method)
see Figs. 30—33.

Fig. 17.

Riehl's melanosis.
Oxygen reduction staining
(Unna's method)
see Figs. 60, 61.

Fig. 18.



Nevus cell nest.
(Gitterfaser staining)

Fig. 19.

Disseminated pigmented spots of Recklinghausen's disease.

Fig. 20.

Same case as Fig. 19.

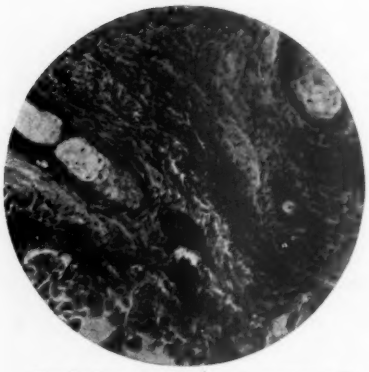
Fig. 21.

Same case as Fig. 19.
The symmetrical malformation of both fourth toes.

PLATE VI

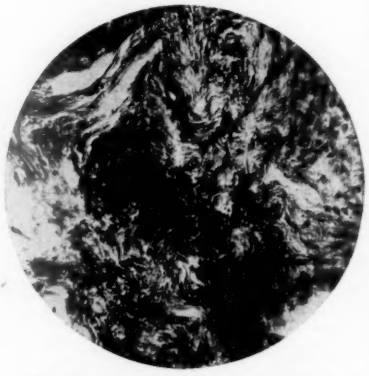
Sections taken from the same case as Fig. 19.

Fig. 22.



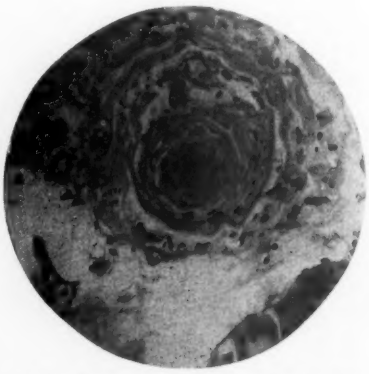
Fascicular structure of nevus cell presenting a marked cellularity. (H.—E.)

Fig. 23.



Fascicular structure of nevus cell presenting an advanced fibrillization. (H.—E.)

Fig. 24.



A drawing of a fibrous nodule in the cutis resembling corpuscule nævique. (van Gieson)

Fig. 25.

A drawing of increased Schwannian cells in the peripheral nerve fiber of the cutis. (H.—E.)

Fig. 26.

Blue nevus, mild type.
(H.–E.)

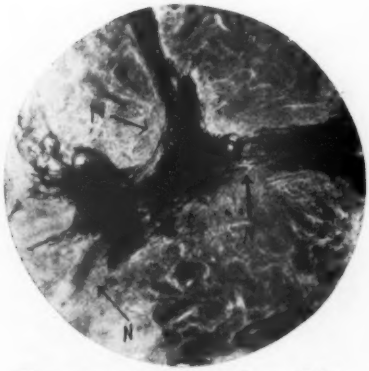
Fig. 27.

Blue nevus, intensive type.
(Silver impregnation)

Fig. 28.

Blue nevus at the deeper layer of the cutis. N.....nerve bundle
(Neurofibril staining)

Fig. 29.



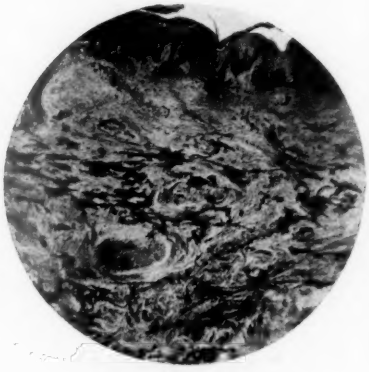
Blue nevus at the deeper layer of the cutis. N.....nerve bundle
(Neurofibril staining)

PLATE VIII

Fig. 30.

Nevus fusco-cæruleus ophthalmo-maxillaris, intensive type.

Fig. 31.

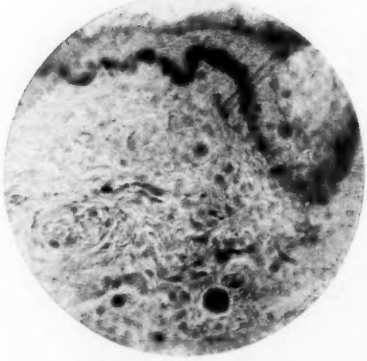


Nevus fusco-cæruleus.
(Silver impregnation)

Fig. 32.

Nevus fusco-cæruleus.
(Neurofibril staining)
Pigment cells runing with the same bend to that of nerve fibers.

Fig. 33.



Nevus fusco-cæruleus.
(Dopa reaction)
Dopa positive cells are seen also in reticulary midlayer.

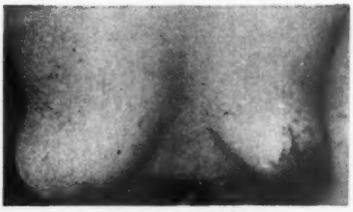
Fig. 34.

Incontinentia pigmenti.
Case 2

Fig. 35.

Incontinentia pigmenti.
Case 3

Fig. 36.



Same case as Fig. 35.
Note the asymmetry of the breast glands.

Fig. 37.

Same case as Fig. 35.

Fig. 38.

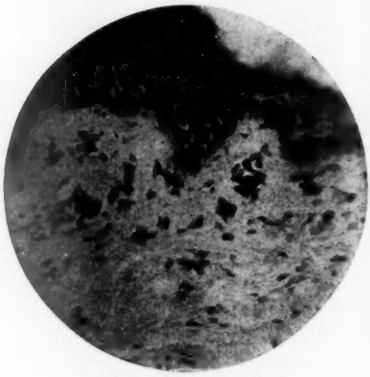
Incontinentia pigmenti achromians.
Note the asymmetry of the breast glands.

PLATE X

Fig. 39.

Incontinentia pigmenti.
Case 3
(H.–E.)

Fig. 40.

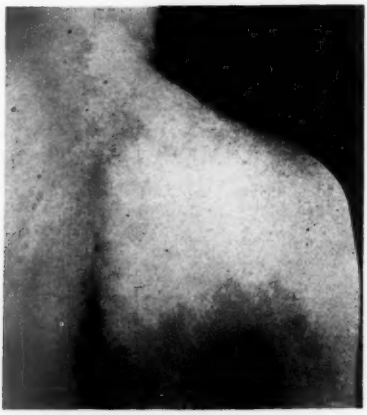


Incontinentia pigmenti.
Case 3
(Silver impregnation)

Fig. 41.

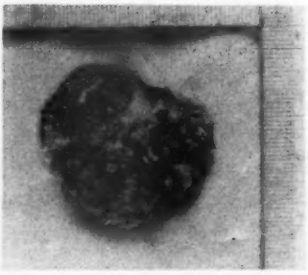
Nevus spilus en nappe.
Case 8

Fig. 42.



Nevus depigmentosus en nappe.
Case 1

Fig. 43.



Melanocarcinoma of the sole.

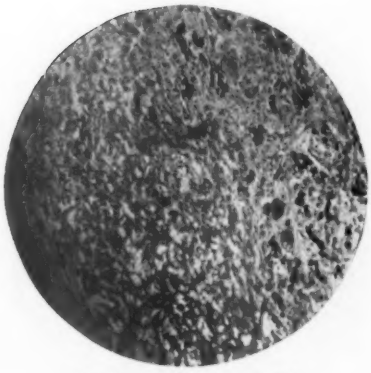
Fig. 44.

Section of Fig. 43. (H.–E.)
T.....Thèque

Fig 46.

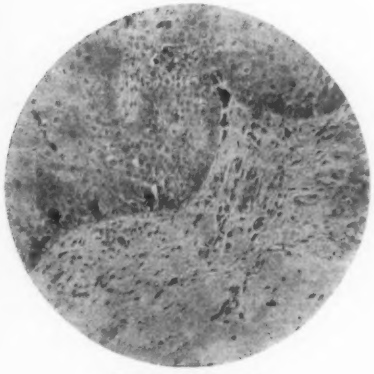
Melanoma of the lip.

Fig. 45.



Section of Fig. 43. (H.–E.)
left...mélanome achromique

Fig. 47.



Section of Fig. 46.
(H.–E.)

PLATE XII

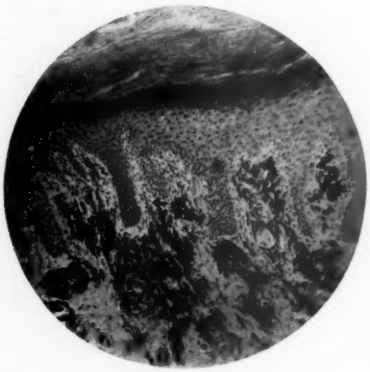
Fig. 48.

Halfring-shaped melanoma-like lesion in a case of carcinoma of the pudenda.

Fig. 49.

Panaris mélaniques.

Fig. 50.



Section of Fig. 49.
(Silver impregnation)

Fig. 51.

Section of Fig. 49.
(Gitterfaser staining)

Fig. 52.

Dark brown islets at regular intervals on linea mediana posterior in leucoderma on lumbar region.

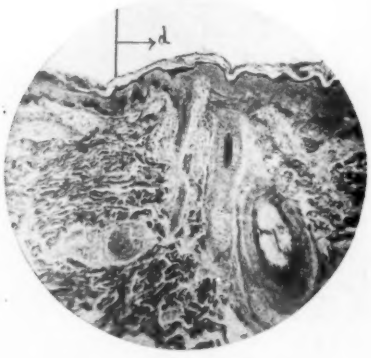
Fig. 53.

Depigmented spots appeared symmetrically on both sides of linea mediana posterior.

Fig. 54.

Sutton's Leucoderma.
Nevus cell nest in the center. (H.–E.)

Fig. 55.



Vitiligo.
Dilatation of blood vessel (v) in the neighbourings of depigmented area(d).
(H.–E.)

PLATE XIV

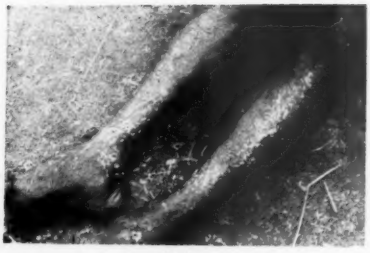
Fig. 56.

Dyschromatosis symmetrica hereditaria.

Fig. 58.

Dyschromatosis following pernio.

Fig. 57.

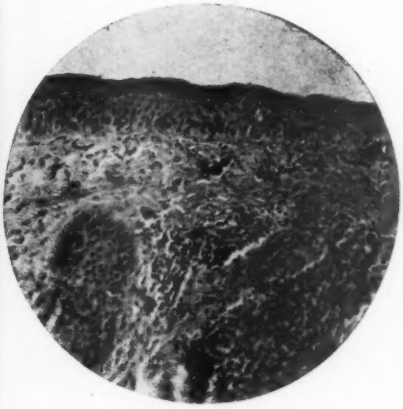


Safu.

Fig. 59.

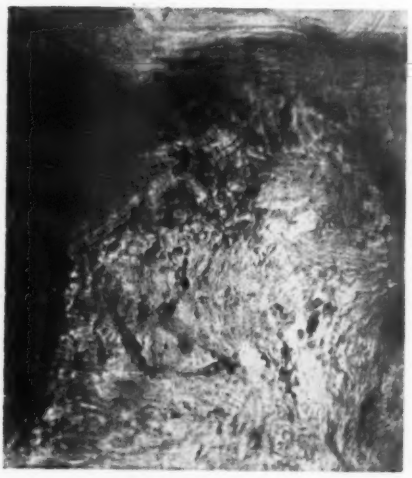
Riehl's melanosis.

Fig. 60.



Riehl's melanosis.
(H.-E.)

Fig. 61.



Riehl's melanosis.
(Dopa reaction)

Fig. 62.

Normal rabbit skin. (Sudan III)

Fig. 63.

Rabbit skin after applying bergamot-oil. (Sudan III)

Fig. 64.

Rabbit skin after vitamin C injection, bergamotoil application and ultra-violet irradiation. (Sudan III)

Fig. 65.

Oxygen reduction staining of normal rabbit skin.

Fig. 66.

Oxygen reduction staining after applying bergamotoil.

Fig. 67.

Oxygen reduction staining after vitamin C injection, bergamotoil applying and ultraviolet irradiation.

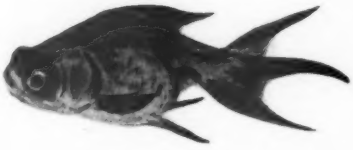
Fig. 68.

Black goldfish *Kurodemekin.*

Fig. 69.

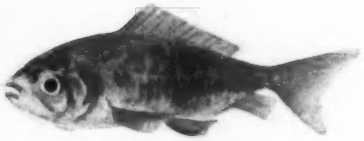
Black goldfish after 7th injection of hydroquinone.

Fig. 70.



Black goldfish after 10th injection of hydroquinone.

Fig. 71.



Yellow-red goldfish

Fig. 72.

Yellow-red goldfish after 6th injection of *p*-benzoquinone.

Fig. 73.

Yellow-red goldfish, 45 days after 14th injection of *p*-benzoquinone.

Fig. 74.

White goldfish with yellow-red spot.

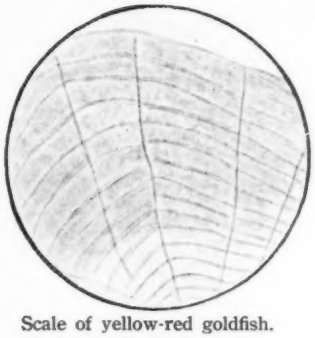
Fig. 75.

White goldfish with yellow-red spot after 12th injection of *p*-benzoquinone.

Fig. 76.

White goldfish with yellow-red spot after 6th injection of hematoporphyrin and ultraviolet ray irradiation.

Fig. 77.

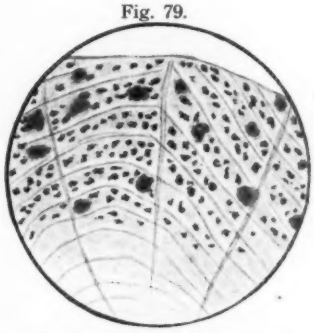


Scale of yellow-red goldfish.
(native preparation)

Fig. 78.

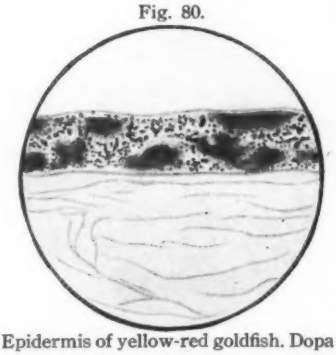
Epidermis of yellow-red goldfish.
(native preparation)

Fig. 79.



Scale of yellow-red goldfish. Dopa reaction after 4th injection of *p*-benzoquinone.

Fig. 80.

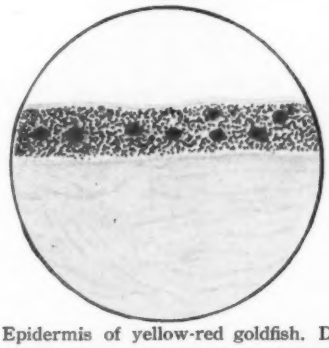


Epidermis of yellow-red goldfish. Dopa reaction after 4th injection of *p*-benzoquinone.

Fig. 81.

Scale of yellow-red goldfish after 10th injection of *p*-benzoquinone.
(native preparation)

Fig. 82.



Epidermis of yellow-red goldfish. Dopa reaction after 10th injection of *p*-benzoquinone.

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